CANADIAN THESES
ON MICROFICHE

30815

THÉSES CANADIENNES
SUR MICROFICHE

AUTHOR/AUTEUR

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TITLE/TITRE

TAXONOMY AND ECOLOGY OF THE GENUS EIMERIA...

THE UNIVERSITY OF ALBERTA

TAXONOMY AND ECOLOGY OF THE GENUS *EIMERIA* (PROTOZOA, EIMERIIDAE) IN THE SNOWSHOE HARE OF CENTRAL ALBERTA

by

HOWARD PAUL SAMOIL ©

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF MASTER OF SCIENCE

DEPARTMENT OF ZOOLOGY

EDMONTON, ALBERTA
FALL, 1976

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled "Taxonomy and Ecology of the Genus *Eimeria* (Protozoa, Eimeriidae) in the Snowshoe Hare of Central Alberta" submitted by Howard Paul Samoil in partial fulfilment of the requirements for the degree of Master of Science.

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ABSTRACT

Nine species of the genus *Eimeria* (Protozoa, Eimeriidae) were found during the examination of 629 snowshoe hares, *Lepus americanus* from central Alberta. Four, *E. robertsoni, E. leporis, E. ruficaudati,* and *E. townsendi*, were described previously from other leporids and are redescribed, while five, *E. athabascensis, E. keithi, E. holmesi, E. rochesterensis* and *E. rowani* are described as new species. The nine species are part of four morphologically similar species groups; they are distinguished on the basis of morphology, presence or absence of oocyst and sporocyst residua and a micropyle, and statistical analysis of oocyst and sporocyst measurements. The eimerians of all lagomorphs are reviewed and a key to the nineteen species from *Lepus* spp. is presented.

Results of cross-transmission studies with *Eimeria* spp., as determined by a review of the literature, suggest that *Sylvilagus* and *Oryctolagus* are more closely related to each other than they are to *Lepus*. Examination of the structural characteristics of *Eimeria* from rodents artiodactyls, and lagomorphs did not reveal any clear host relationships.

Overall prevalences of from 5 to 50% were recorded for the nine species. All nine species were observed in neonatal hares approximately one week old. The oocyst output of seven species decreased with increasing age of the hares. Seasonal factors were important modifiers of oocyst output for five of the nine species. Sex and host density did not appear to influence patterns of either prevalence or intensity.

ACKNOWLEDGMENTS

I would like to thank my supervisor Dr. W. M. Samuel for his advice, encouragement and patience throughout the course of this study, and for his valuable editorial assistance in the preparation of this thesis.

I am indebted to the personnel of the Rochester Wildlife Research Center, especially graduate students John Carey, Mike Vaughn, Chris Brand, Lamar Windberg and the Director, Dr. L. B. Keith.

I would also like to thank Brian Lajeunesse and especially Doug Wing for their assistance in the field. Thanks are also due to student members of the Parasitology group of the Department of Zoology for their friendship, help and argument throughout this study. Thanks are due also to technicians Marilyn Martin, Doris Harder and Bob McClymont for their assistance.

Thanks are extended to Dr. John Addicott for the program and technical assistance that resulted in most of the figures of the ecology section. A special thanks to Margaret Bush for her excellent work on the figures in the taxonomy section. Thanks are also due to Margaret Bush, Al Bush, Jonna Samuel and Judy Samoil for assistance in the preparation of the text, tables and figures. A special thanks to Mrs. Minnie Cutts for her excellent work on the typescript.

This study was supported in part by funds from the National Research Council of Canada (Grant A-6603 to W. M. Samuel), the Boreal Institute for Northern Studies, and the University of Alberta.

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I. INTRODUCTION

Although many species of coccidia have been reported from representatives of the Order Lagomorpha (Hobbs and Samuel 1974 and reviews of Pellérdy 1974, and Levine and Ivens 1972), no one has studied this group of parasites in the snowshoe hare (*Lepus americanus*) in any detail. MacLulich (1937) mentioned that coccidia were present in hares from Ontario, but no species were identified. In the only other report on the subject, Boughton (1932) listed *Eimeria stiedai* (Lindemann 1865) and *Eimeria perforans* (Leuckart 1879) from the snowshoe hare. The validity of his findings has been questioned by Carvalho (1943) and Pellérdy (1974). The criticisms were based on the fact that Boughton identified *E. stiedai* only after examination of apparently unsporulated oocysts in the feces; he did not examine the contents of the gall bladder and bile ducts, unusual locations for eimerians, but the normal locations for *E. stiedai*. Unsporulated oocysts of *E. stiedai* resemble closely at least one other species, *E. magna* Perard 1925, of the intestinal tract of Lagomorpha, so the criticisms have merit.

Pellérdy (1974) suggested that *E. perforans* is found only in *Oryctolagus cuniculus* and possibly members of the genus *Sylvilagus*, thus he refuted Boughton's finding on the basis of host specificity. Levine and Ivens (1972) added, "At one time all species of *Eimeria* in the lagomorph intestine were thought to be *E. perforans*. As a result, earlier reports of this species in cottontails and hares must be discounted." The descriptions of Boughton (1932) are incomplete and give no good clues to the real identity of the coccidia recovered.

The sparse literature on coccidia of *Lepus americanus* is in contrast to that for the wild rabbit, *Oryctolagus cuniculus*, in New Zealand and Australia, and for cottontails of the genus *Sylvilagus* in North America. The number of species of coccidia and their identification in both hosts have received considerable attention (for *Oryctolagus* see Kessel and Jankiewicz 1931, Rutherford 1943, Pellérdy 1974; for *Sylvilagus* see Honess 1939, Carvalho 1942, 1943, 1944, Herman and Jankiewicz 1943, Duszynski and Marquardt 1969). These studies rely heavily on descriptive morphology to identify the species encountered. There are two noteworthy exceptions: the works of Carvalho (1942, 1943, 1944), which utilized cross-transmission experiments as an aid to the identity of species, and Duszynski and Marquardt (1969), who used a statistical approach to help in their taxonomic decisions.

As a consequence of the taxonomic work on coccidia of *Oryctolagus* and *Sylvilagus*, other studies on the nature of the coccidia-host relationship have been conducted. The most intensive research has focused on the relationships between eimerian infections and age and sex of the wild rabbit (*O. cuniculus*), and the influence of season and climatic region in Australia (Mykytowycz 1962, Stodart 1968a, 1968b, 1971, Dunsmore 1971) and New Zealand (Bull, 1958). Ecologic studies on *Eimeria* of *Sylvilagus* has been restricted to brief analysis of winter coccidiosis (Dorney 1962) and the role of coccidiosis in host mortality (Ecke and Yeatter 1956).

The initial and primary objective of the present study was to investigate several aspects of the ecology of coccidia of the snowshoe hare (*Lepus americanus*) with particular emphasis on the demography of the host and parasites over time. However, based on the history of similar studies on *O. cuniculus* and taxonomic problems that became immediately obvious, an initial study on the taxonomy of the coccidia was conducted. Its objectives were:

(1) to determine the identity, prevalence, and intensity of *Eimeria* spp. in a population of snowshoe hares from central Alberta;

(2) to present a detailed morphological description or redescription (if necessary) of each species observed including drawings, photomicrographs, and pertinent statistical data;

(3) to present a workable taxonomic key to the *Eimeria* of the snowshoe hare;

(4) to discuss the relationships of the coccidia of the genus *Lepus* with other members of the Order Lagomorpha, and with coccidia of other mammalian orders;

(5) to examine the use of coccidia as indicators of the phylogenetic relationships of members of the Order Lagomorpha, and with other orders of mammals.

With the basis provided by the taxonomic investigation, some aspects of the hare-*Eimeria* relationship were examined. Sprent (1963) considered the host-parasite relationship to be "an equilibrium of two biological factors" which could be influenced by the "environment". Sprent's "environment" was composed of two segments: the external, where climatic and geographical factors affect the survival of both the parasite and the host; and the internal, which is the host itself.

Some of the factors which may influence the internal environment are age, sex and social status of the host. Several workers (Bull 1958, Mykytowycz 1962, Stodart 1968a, 1971) have found a decrease in intensity of infection by *Eimeria* with increasing age of the rabbit, *O. cuniculus*.

Season and social status of the host also influenced oocyst output at least with *O. cuniculus* in enclosures (Mykytowycz 1962), as did climatic and geographical factors in free-living populations (Stodart 1968a, 1968b, 1971).

As a result of the work done with *Oryctolagus* and the availability of large numbers of snowshoe hares, one of the objectives of the ecology portion of the present study was to investigate selected aspects of the internal environment such as age and sex of the host. A second objective was to examine the effect of the external environment indirectly by determining the seasonal patterns of prevalence and intensity of the species of *Eimeria* observed.

Populations of the snowshoe hare undergo periodic fluctuations in density usually termed a "ten-year cycle" (Keith 1963). One of the characteristics of these cycles is a higher juvenile mortality during the decline phase. Coccidiosis has been implicated as a cause of juvenile mortality in *O. cuniculus* in Australia (Dunsmore 1971) and New Zealand (Bull 1958). Therefore, since the present study took place during the decline phases of the "ten-year cycle", the final objectives of this study were to examine the effect of varying host densities on the host-parasite relationship and to determine if coccidiosis was involved in juvenile mortality.

II. STUDY AREA

The study area of approximately 2,500 km^2 lies in north central Alberta, 100 km. north of Edmonton (54N, 113W). The climate is continental with warm summers and cold winters. The area receives 40 to 45 cm. of precipitation a year, with two-thirds of that coming between May and September (Kjearsgaard 1972). The terrain is flat to gently rolling, with the exception of the Tawatinaw River Valley. Although some land has been cleared for agriculture, most of the area is lowland bog with tamarack (*Larix laricina*) and black spruce (*Picea mariana*) and upland with balsam poplar (*Populus balsamifera*), aspen (*Populus tremuloides*), paper birch (*Betula papyrifera*), jackpine (*Pinus banksiana*) and white spruce (*Picea glauca*). The study area is within the area designated by Moss (1955) as the Mixed Wood Section of the Boreal Forest Region. Further information on the vegetation, climate, physiography, and soil formations of the area are given by Moss (1955), Meslow and Keith (1968), Weatherill and Keith (1969), Rusch *et al.* (1971), and Kjearsgaard (1972).

III. MATERIALS AND METHODS

Fecal samples were taken from 629 hares collected between May 1, 1971, and August 31, 1972, near Rochester, Alberta. Hares were trapped in many locations by personnel of the University of Wisconsin Wildlife Research Center at Rochester as part of a long-term study of population dynamics of the snowshoe hare. The Center, under the supervision of Dr. L. B. Keith, Department of Wildlife Ecology, University of Wisconsin, has been collecting data on the population demography of snowshoe hares since 1961.

Animals were live-trapped, brought to the Center, killed, and necropsied. Information collected from each hare included sex, reproductive status of both males and females, entire weight, paunched weight, hind foot length, and weights of various organs (testes, adrenals, heart, liver, and spleen). In addition, eye lenses were taken and processed according to Keith *et al.* (1968) to provide an estimate of age. The sex and age structure of the sample used in the present study is provided in Table 1. Animals less than one year of age were classed as juveniles, animals in their first breeding season were classed as yearlings, and animals in their second or subsequent breeding season were classed as adults. Neonatal hares were aged to the nearest week according to the lens weight curves of Keith *et al.* (1968). A condition index was calculated using the formula of Brand *et al.* (1975). The techniques for the population studies were described in detail by Keith *et al.* (1968).

Fecal samples, collected from the rectum freshly killed snowshoe hares, were placed in vials containing approximately 10 ml. of 2.5% aqueous

Table 1. Sex and age composition of snowshoe hares collected during eight periods (1971-1972)

SEX AND AGE CLASS	COLLECTING PERIODS								TOTAL
	1 (May 1 - May 15, 1971)	2 (May 16 - July 15, 1971)	3 (July 16 - August 31, 1971)	4 (Sept. 1 - Oct. 31, 1971)	5 (Nov. 1, 1971-Mar. 31, 1972)	6 (April 1 - May 15, 1972)	7 (May 16 - July 15, 1972)	8 (July 16 - August 31, 1972)	
Juveniles	3	31	44	13	30	10	6	6	143
Yearlings	4	46	24	3	19	5	15	2	118
Adults	2	13	5	0	10	10	16	1	57
Total Males	9	90	73	16	59	25	37	9	318
Juveniles	4	32	29	11	38	13	7	4	138
Yearlings	6	31	20	1	17	8	11	2	96
Adults	8	22	7	4	6	17	12	1	77
Total Females	18	85	56	16	61	38	30	7	311
GRAND TOTAL	27	175	129	32	120	63	67	16	629

potassium dichromate ($K_2Cr_2O_7$) solution. After storage for varying periods of time (usually less than one week) at 4C, the vials were uncapped and the samples left to sporulate at 20C for seven to ten days. Then they were either examined for coccidia or stored at 4C for examination later. Sporulation times were determined from fresh, macerated feces, placed in 100 X 15 mm. Petri dishes (with the depth of the solution not exceeding 1 cm.) and left at room temperature for seven days. These samples were stirred twice a day.

Oocysts were concentrated for study by flotation using the technique and modification of Samuel and Trainer (1969) and Samuel and Gray (1974), respectively. However, since the fecal sample was not weighed prior to placement in potassium dichromate, it was necessary to introduce the following modification. A portion (approximately 5 ml.) of each fecal-potassium dichromate mixture was poured through cheese cloth into a 125 X 16 mm. graduated centrifuge tube. The sample was centrifuged for 10 minutes at 1,100 rpm., the packed volume of feces recorded, and the supernatant poured off. Approximately 6 ml. of sugar solution (sp. gr. 1.27) was added and mixed with the packed feces. Sugar solution was added until a positive meniscus was formed above the lip of the tube. A 22-mm. square coverslip was placed on the centrifuge tube and the sample centrifuged for 10 minutes at 1,100 rpm. The coverslip was lifted off briskly and placed on a glass slide. Each coverslip was examined in its entirety for coccidia using a 10X eyepiece and a 20X objective with a total count of the oocysts present being made. All oocysts were identified if there were less than 100 oocysts present, and at least 100 were chosen at random and identified if more than 100 were present. In order to determine the number of oocysts for each species in those samples with more than 100 oocysts the total count was multiplied by the proportion of each species.

The following procedure was used to get the number of oocysts per gram of feces. The volume of packed feces was used in the following equation to determine the weight of the fecal sample:

$$Y = 0.018 + 0.11X$$

$(r = 0.96, P \leq 0.05)$

where Y is the volume of feces in ml., and X is the weight of feces in grams.

The above relationship was determined by a series of runs (a total of 27 samples) in which varying amounts of the fecal-dichromate mixture (0.2 ml. to 2.5 ml.) were centrifuged for 10 minutes at 1,100 rpm., the supernatant removed and the fecal sample air dried at 30C. The samples were weighed every day until there was no change in weight on two consecutive days.

All oocysts examined and measured for descriptions were from freshly sporulated material. Oocysts were examined with a Wild M20 compound microscope using 100X apochromatic oil immersion objectives with 10X eyepieces and measured with an ocular micrometer. Composite drawings were made using the camera lucida; photomicrographs were taken using Kodak Pan-X film and a Zeiss photomicroscope.

Thirty oocysts and sporocysts were measured from at least two hares for three species (*E. rowani* sp. n., *E. rochesterensis* sp. n., and *E. holmesi* sp. n.) while 100 oocysts and sporocysts were measured from at least four hares for the remaining six species.

Data collected from hare fecal examinations were grouped into eight collecting periods for analysis (Table 1). These periods reflect climatic seasons in central Alberta as well as biological changes in the snowshoe hare. The winter period, November 1 to March 31, is characterized by low temperatures (range -20 to 0C), snow cover, short day length and a change in the animal's diet to a heavy utilization of woody browse (Dodds 1960). The spring period, April 1 to May 15, has a temperature range of -6 to 16C, increasing day length and light intensity, changes in the diet of the snowshoe hare to herbaceous material as it appears (Meslow and Keith 1971), and initiation of breeding. Summer was subdivided into two periods. The first, May 16 to July 15, constitutes the breeding season; that is, females are pregnant, males are sexually active, and young are born. The second is a period when the testes of the adult males regress, adult females are recovering from parturition, and juveniles undergo rapid growth. Temperatures in summer range from 6 to 28C, day length is long, and hares utilize herbaceous vegetation exclusively. The fall period, September 1 to October 31, is characterized by a temperature range of 16 to -2C, shortening day length, and a changeover to woody browse from herbaceous vegetations.

Statistical analysis of the data was done on an IBM 360 computer using APLSV or SPSS. Programs used were obtained from the public library of the University of Alberta Computing Center and were based on procedures outlined in Nie *et al.* (1975), Sokal and Rohlf (1969), and Steel and Torrie (1960).

The data were tested for normality of distribution using a program for the Kolmogorov-Smirnov test. The data on oocyst counts were normalized using the transformation $\log_{10}(X + 1)$ where X equals the number of oocysts per gram of feces. Most of the data were analyzed by the analysis of variance (SPSS: ANOVA). Linear regressions of the oocyst count of each species on eye lens weights were computed using the SCATTERGRAM program in SPSS. Other methods of analysis of the data are given when first used.

IV. TAXONOMY

Introduction

There are three biological characteristics which are used to distinguish the species of coccidia: oocyst morphology, host specificity, and location and timing of the endogenous stages. Oocyst morphology and host specificity usually become the bases for species identification, since the location and timing of the endogenous stages is known for only a few species (see Levine and Ivens 1965, 1970). Host specifity, as determined by cross-transmission experiments, is also poorly known (Levine and Ivens 1965, 1970); it is often combined with information on the geography of hosts with eimerians of similar morphology to determine the identity of species.

Marquardt (1973) made the following statement about the practices of the authors describing new species of coccidia:

> In describing new species, authors have generally held to the principles that oocysts of identical morphology from closely related hosts are members of a single species. As a corollary, it has also been concluded that oocysts of similar structure from hosts that are widely separated taxonomically are different species. Likewise oocysts of different structures but derived from the same host are different species.

This statement implies two questions which all parasite taxonomists must answer before describing a new species. The first is what degree of variability is inherent in the parasite species. The second concerns the degree of host specificity; what are "closely related" and "widely separated" host taxa as far as the parasite is concerned. A discussion of the answers to these questions with regard to the coccidia of the snowshoe hare is warranted.

The morphological characters used in distinguishing the oocysts of different species are size, shape, color, and texture of the oocyst and sporocyst walls, presence or absence of an oocyst micropyle, polar body, and residuum, and presence or absence of a sporocyst residuum. Some of these characteristics have been shown to vary for one or more species of coccidia. Changes in oocyst size during the patent period have been recorded often (see review of Duszynski 1971, Samoil and Samuel, Appendix I). Kheysin (1957) has shown that shape of the oocyst may vary with the size of the initial infective dose and the time of the patent period. The color of the oocyst wall may vary depending on the type of lens or filters used on the microscope. The size of the oocyst and sporocyst residua has been shown to decrease with storage (Duszynski and Marquardt 1971, Kheysin 1959, 1967).

Thus, although several characters vary in size or color, their presence or absence is constant. As a consequence, the morphologic characteristics which appear to be most reliable for species identification are: presence or absence of a micropyle, oocyst residuum, sporocyst residuum, and oocyst polar body. Size, although it has been shown to vary, can be used as a tool for identification of species if the variation is documented and when supported by other criteria, particularly that related to presence or absence of characters (Jones 1932). Shape of the oocyst, although it varies within limits similar to size, may also be used in conjunction with other criteria as a species characteristic

(Kheysin 1957).

In this study species of *Eimeria* were distinguished using the above characteristics. Freshly sporulated material was examined to determine the presence or absence of a micropyle, oocyst polar body, oocyst residuum, and a sporocyst residuum. Although there was a decrease in size of the oocyst and sporocyst residua for some species from fecal material which had been stored in potassium dichromate for up to two years, the presence or absence of these characters was not affected. The descriptions were based on oocysts from different animals and different oocyst densities so as to encompass the greatest range of variability in size for each species.

Answers to the second question (that is, what is the degree of host specifity?) can provide more than just information on the identity of the parasite species. The use of parasites as clues to the evolution and phylogenetic affinities of hosts has been examined by numerous authors (Metcalf 1929, Janiszewsko 1949, Cox 1967). Janiszewsko (1949) proposed the term "parasitogenesis" to refer to the evolution of relationships between the parasite and its host. Several "rules of affinity" have been proposed (see Dogiel 1964), with the major one postulating the parallel evolution of the parasites and their hosts. Hennig (1966) suggested that these rules have many exceptions and, therefore, they should be used only as guides for the study of phylogenetic relationships. He stated further that even in its imperfect state the parasitological method has helped to clarify some obscure phylogenetic relationships.

Metcalf (1929), in a perceptual paper, examined the aid parasites give in solving problems of host taxonomy and biogeography. He suggested that the sporozoa (*Eimeria, Plasmodium,* etc.) presented a good opportunity for such studies, due to their high degree of host specificity. In spite of this suggestion, little use has been made of this group to elucidate phylogenetic relationships, and workers have concentrated mainly on arthropods (Clay 1957, Hopkins 1957a, 1957b) and trematodes (Llewellyn 1957, Manter 1957).

Given this information and the fact that the genus *Eimeria* is common and has been studied taxonomically in many mammals, and is thought to be host specific for "widely separated" taxons, I decided to examine Metcalf's (1929) hypothesis in some detail. The primary objective was to examine the relationships of various genera of the Lagomorpha and their eimerians as well as comparing the eimerians of lagomorphs with those of other host taxons in an attempt to clarify phylogenetic affinities of the lagomorphs.

Results

Nine distinct types of oocysts (Protozoa: Eimeriidae: *Eimeria*) were observed during this study and compared with those already described from lagomorphs and other higher mammalian taxons (see reviews of Levine and Ivens 1972, Pellérdy 1974). The original descriptions or redescriptions of four of the nine species were incomplete; the four, *E. robertsoni* (Madsen 1938) Carvalho 1943, *E. leporis* (Nieschulz 1923), *E. ruficaudati* Gill and Ray 1960 and *E. townsendi* (Carvalho 1943) Pellérdy 1956, are redescribed here. The redescriptions contain an indication of the variability of character measurements, the lack of which is the major problem with previous descriptions. Specifically, the number of oocysts measured, mean and range of the measurements and a statistic, the standard error of the mean, have been provided. The remaining five, *E. athabascensis*, *E. keithi*, *E. holmesi*, *E. rochesterensis*, and *E. rowani* are described as new species.

Eimeria townsendi, *E. robertsoni*, and *E. holmesi* were easily distinguished on the basis of their morphology, but the only observable differences among the remaining six were the presence or absence of oocyst and sporocyst residua and a micropyle. Therefore, these forms were subjected to simple statistical analysis to determine if any size differences were significant. All measurements are in micrometers, with means and standard errors in parentheses following the range.

Description of the Parasites

Phylum	Protozoa	Goldfuss 1818 emend. von Siebold, 1845
Subphylum	Sporozoa	Leuckart 1879
Class	Telosporea	Schaudinn 1900
Subclass	Coccidia	Leuckart 1879
Order	Eucoccidia	Leger and Duboscq 1910
Suborder	Eimeriina	Leger 1911
Family	Eimeriidae	Poche 1913
Genus	*Eimeria*	Schneider 1875

****

Eimeria robertsoni (Madsen 1938) Carvalho 1943

(Figures 1, 11)

Synonyms: *Eimeria magna* var *robertsoni* Madsen 1938, *Eimeria magna* forma *townsendi* Carvalho 1943 pro parte, *Eimeria perforans* var *groenlandica* Madsen 1938.

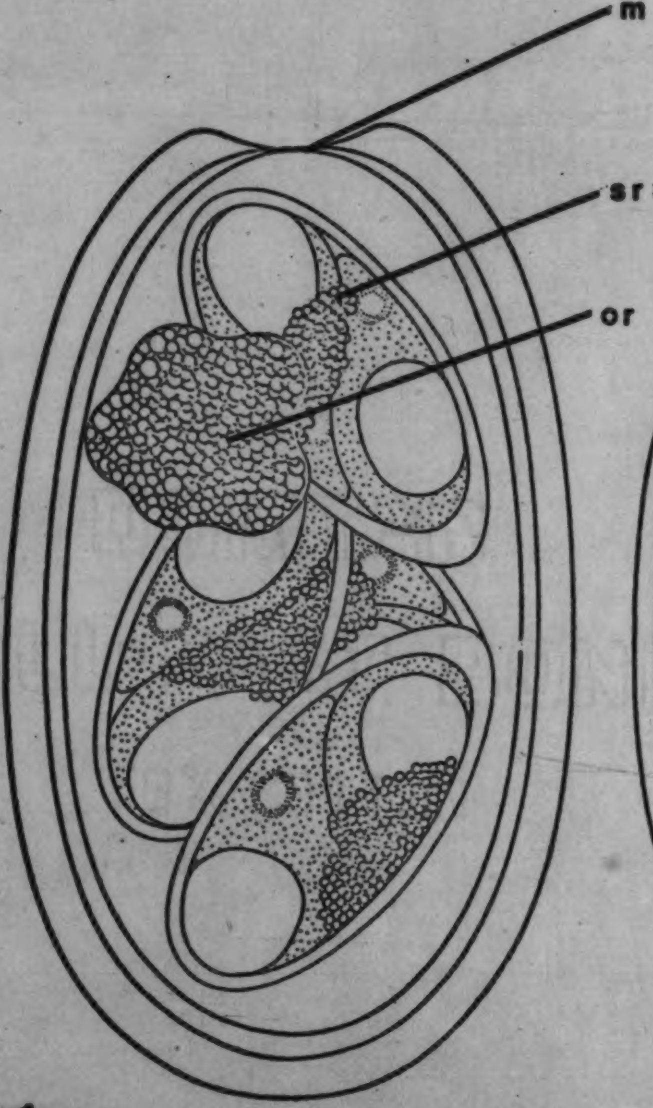
Type host: *Lepus arcticus.*

Other hosts: *Lepus townsendii, Lepus europaeus, Lepus ruficaudatus, Lepus timidus, Lepus americanus.*

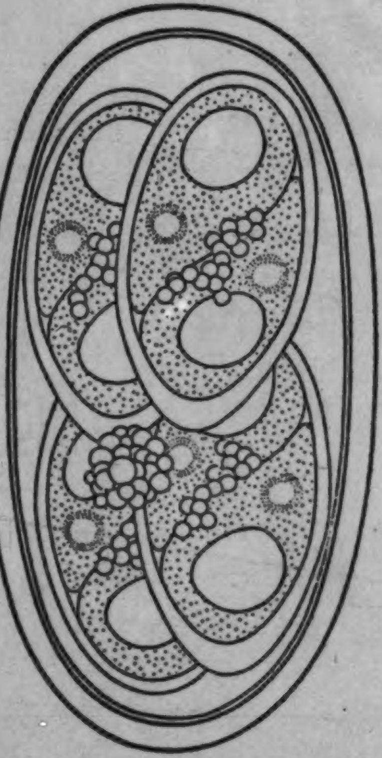
Redescription: Sporulated oocysts broadly ellipsoid or ovoid, with a broad micropyle from 4.8 to 6.0 (5.9 ± 0.1) wide; oocyst wall consisting of two layers, the outer yellowish-brown, finely granular, easily detached, 1.4 thick; the inner 0.5 thick; 100 sporulated oocysts 33.6 to 51.6 (41.3 ± 0.4) long, and 20.4 to 25.2 (22.5 ± 0.1) wide; polar granule absent; oocyst residuum compact, granular and large, 6.0 to 10.8 (8.1 ± 0.1) in diameter; sporocysts elongated spindle-shaped bodies, tapering toward one end, 18.0 to 25.2 (20.9 ± 0.2) long and 6.0 to 9.6 (8.0 ± 0.1) wide; Stieda body present as an indistinct light refracting body at the narrow end (not apparent in Figure 11, not shown in Figure 1); diffuse granular sporocyst residua present; sporozoites with a single refractile body posterior to the nucleus.

Figure 1. Sporulated oocyst of *Eimeria robertsoni* (m = micropyle, or = oocyst residuum, sr = sporocyst residuum)

Figure 2. Sporulated oocyst of *Eimeria leporis*



1



2

20 µm

Sporulation time: Most oocysts were sporulated after 6 days at 20C.

Range: North America, Europe, Greenland, India.

Location in host: Duodenum (Carvalho 1943).

Percentage infection: 208 of 629 (33.1%) snowshoe hares of central Alberta.

Remarks: This description of *E. robertsoni* differs slightly from those of Madsen (1938), Carvalho (1943), Pellérdy (1956), Levine and Ivens (1972). The oocyst wall is two-layered; the outer layer was removed easily with 5% sodium hypochlorite solution. The text of all four previous descriptions (op. cit.) indicates only a single layer, although the drawings in three (Madsen, Pellérdy, and Levine and Ivens, op. cit.) show two layers. The sporulation time of oocysts in the present study was six days, which agrees with results of Bouvier (1967), but not those of the other describers mentioned previously. The sporocyst residua are large in freshly sporulated oocysts, but markedly smaller and not always visible in all sporocysts in oocysts which have been stored for periods of up to two years. Kheysin (1967) has reviewed the literature on the decreasing size of the sporocyst residua, on storage concluding that there is a nutritive material within the residua which is used by the sporozoites. This description also differs from previous ones in that a Stieda body is reported for the first time.

Aspects of the biology of this species (patent period, oocyst output during the patent period, and changes in the size of the oocyst during the patent period) have been reported on by Samoil and Samuel (unpublished data in Appendix I).

Eimeria leporis Nieschulz 1923

(Figures 2, 15)

Type host: *Lepus europaeus*.

Other hosts: *Lepus americanus, Lepus arcticus groenlandicus, Lepus ruficaudatus, Lepus timidus, Lepus tolai*.

Redescription: Sporulated oocysts cylindrical, micropyle absent or indistinct if present; oocyst wall thickness about 1, consisting of two layers, the outer smooth and uniformly thick; 100 sporulated oocysts 27.6 to 39.6 (32.1 ± 0.3) long and 12.8 to 16.8 (15.6 ± 0.1) wide; polar granule absent; oocyst residuum compact, granular, and 2.4 to 6.0 (3.9 ± 0.1) in diameter; sporocysts elongated spindle-shaped bodies, tapering toward one end, 11.0 to 18.0 (14.4 ± 0.2) long and 5.8 to 7.2 (6.3 ± 0.1) wide; Stieda body present as an indistinct light refracting area at the narrow end (not apparent in Figure 15, not shown in Figure 2); diffuse granular sporocyst residua present; sporozoites with a single refractile body posterior to the nucleus.

Sporulation time: Most oocysts were sporulated after 6 days at 20C.

Range: Europe, Greenland, India, Canada (Alberta).

Location in host: Small intestine (Nieschulz 1923, Pellérdy *et al.* 1974).

Percentage infection: 223 of 629 (35.5%) snowshoe hares of central Alberta.

Remarks: The present description differs slightly from the previous description of Pellérdy *et al.* (1974) as follows: the sporocyst is larger (11 to 18 compared to 9 to 10), and the sporulation time is longer (6 days at 20C compared to "2 - 3 days at room temperature"). Pellérdy *et al.* (1974) provide no details on the method of sporulation. The present description confirms the presence of a Stieda body, which was first reported by Pellérdy *et al.* (1974).

Eimeria ruficaudati Gill and Ray 1960
(Figures 3, 18)

Type host: *Lepus ruficaudatus.*

Other hosts: *Lepus americanus.*

Redescription: Sporulated oocysts cylindrical, micropyle distinct and sunken slightly into oocyst wall, 3.6 to 6.0 (4.5 ± 0.1) wide; oocyst wall thickness about 1, consisting of two layers, the outer smooth and uniformly thick, being several times thicker than the inner layer; 100 sporulated oocysts 22.8 to 38.4 (29.9 ± 0.3) long and 13.9 to 19.2 (17.5 ± 0.1) wide; polar granule absent; oocyst residuum compact, granular, and 2.4 to 5.8 (4.1 ± 0.6) in diameter; sporocysts elongated spindle-shaped bodies, tapering toward one end, 11.6 to 18.0 (15.2 ± 0.2) long and 5.8 to 8.1 (6.6 ± 0.1) wide; Stieda body present as an indistinct light refracting area at the narrow end (not apparent in Figure 18, not shown in Figure 3); sporocyst residua diffuse and granular sporozoites with a single refractile body posterior to the nucleus.

Sporulation time: Most oocysts were sporulated after 6 days at 20C.

Range: India, Canada (Alberta).

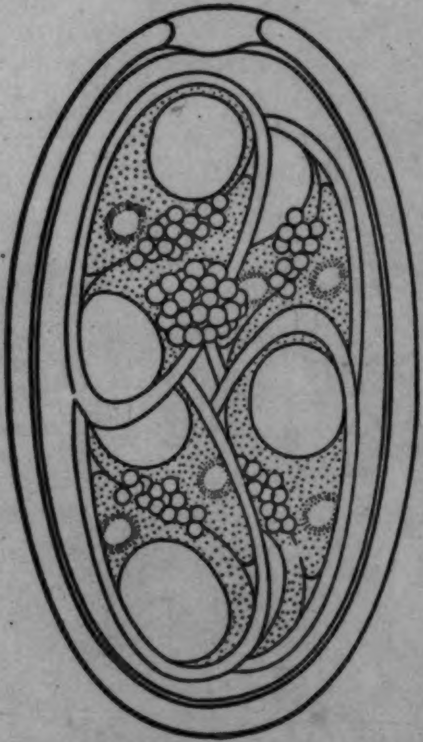
Location in host: Not known, recovered from fecal samples.

Percentage infection: 101 of 629 (16.1%) snowshoe hares of central Alberta.

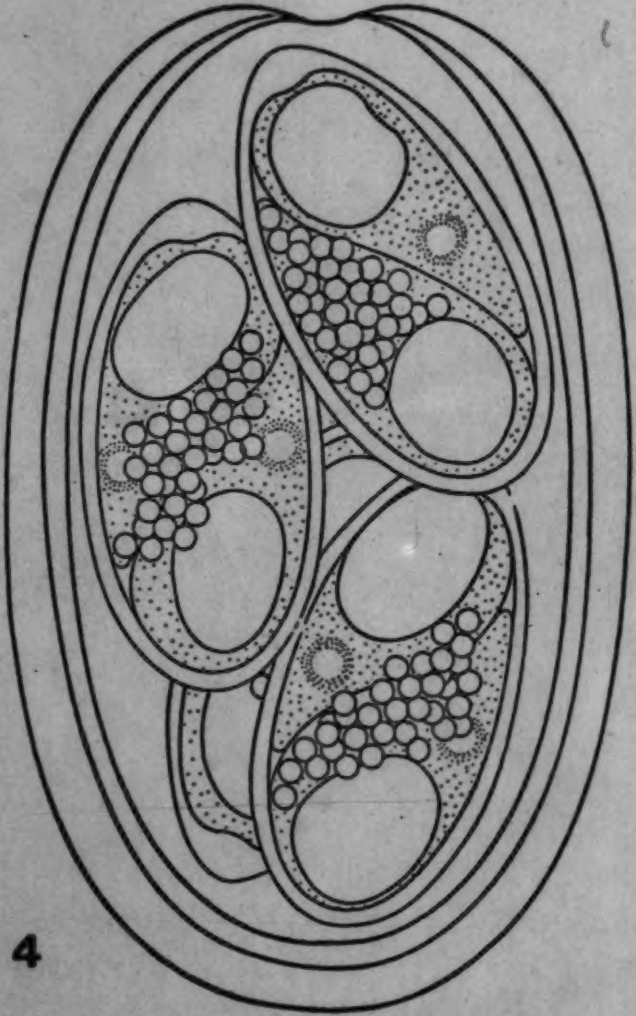
Remarks: This description provides measurements of the micropyle and the sporocysts which are not provided by Gill and Ray (1960). The oocyst width is incorrectly given by Gill and Ray (1960) as

Figure 3. Sporulated oocyst of *Eimeria ruficaudati*

Figure 4. Sporulated oocyst of *Eimeria townsendi*



3



4

20 µm

12 to 15, with an average of 17.5, a value outside the range. Gill and Ray (1960) report the sporulation time as 66 hours, but the temperature at which material was sporulated was 28C. The difference in temperature probably accounts for the difference in sporulation time. They also report the disappearance of the sporocyst residua with "preservation"; the sporocyst residua did not disappear in material used in this study which had been stored for up to two years. A Stieda body is reported for the first time in this species.

Eimeria townsendi (Carvalho 1943) Pellérdy 1956

(Figures 4, 10)

Synonyms: *Eimeria magna* forma *townsendi* Carvalho 1956 pro parte; *Eimeria magna* Perard 1925 pro parte.

Type host: *Lepus townsendii*.

Other hosts: *Lepus timidus*, *Lepus europaeus*, *Lepus americanus*, *Lepus californicus*.

Redescription: Oocysts broadly ellipsoid or ovoid with a broad micropyle from 4.8 to 7.2 (5.6 ± 0.6) wide; oocyst wall consisting of two layers, the outer yellowish-brown, finely granular, easily detached, about 1.4 thick, the inner about 0.5 thick; 100 sporulated oocysts 36.0 to 49.2 (39.6 ± 0.2) long, and 19.2 to 24.0 (22.9 ± 0.1) wide;

polar granule absent; oocyst residuum absent; sporocysts elongated spindle-shaped bodies tapering toward one end, 16.8 to 20.4 (18.4 ± 0.1) long, and 8.4 to 9.6 (9.1 ± 0.1) wide; Stieda body present as an indistinct light refracting area at the narrow end (not apparent in Figure 10, not shown in Figure 4); large diffuse granular sporocyst residua; sporozoites with a single refractable body posterior to the nucleus.

Sporulation time: Most oocysts were sporulated after 6 days at 20C.

Range: Europe, North America.

Location in host: Unknown, although Pellérdy (1974) suggests the small intestine.

Percentage infection: 314 of 629 (49.9%) snowshoe hares of central Alberta.

Remarks: This description differs from previous descriptions in that it presents measurements for the micropyle and reports the presence of a Stieda body. There are minor differences in the width of the oocyst and in sporulation time.

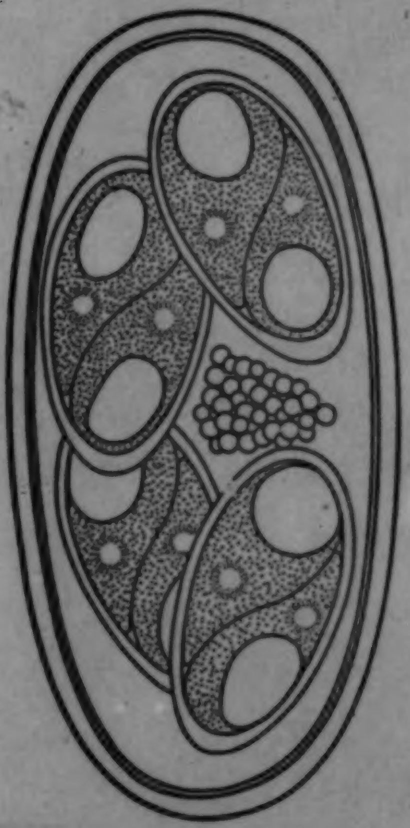
Eimeria athabascensis sp. n.

(Figures 5, 16)

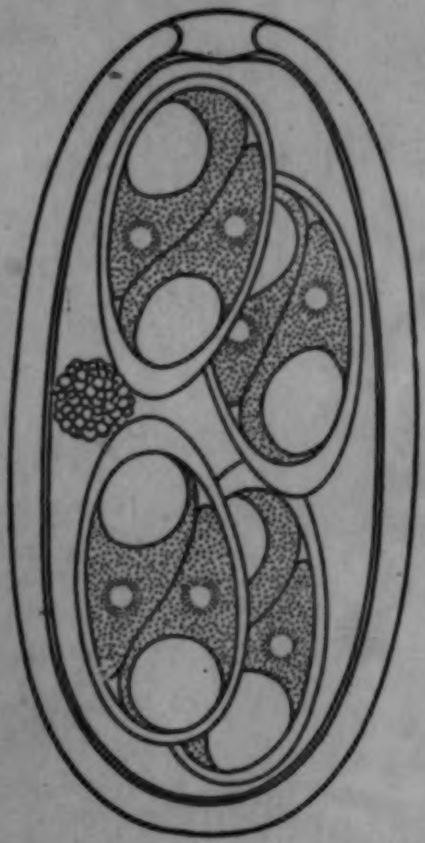
Type host: *Lepus americanus*.

Figure 5. Sporulated oocyst of *Eimeria athabascensis*

Figure 6. Sporulated oocyst of *Eimeria keithi*



5



6

20 µm

Type locality: Rochester, Alberta, Canada.

Description: Sporulated oocysts cylindrical, micropyle absent; oocyst wall thickness about 1, consisting of two layers, the outer smooth and uniformly thick, being several times thicker than the inner layer; 100 sporulated oocysts 24.0 to 38.4 (33.8 ± 0.3) long, and 13.2 to 16.8 (15.6 ± 0.1) wide; polar granule absent; oocyst residuum compact, granular, and 2.4 to 4.8 (3.5 ± 0.1) in diameter; sporocysts elongated spindle-shaped bodies tapering toward one end, 12.0 to 18.0 (15.1 ± 0.1) long and 6.0 to 7.6 (6.2 ± 0.1) wide; Stieda body present as an indistinct light refracting area at the narrow end (not apparent in Figure 16, not shown in Figure 5); sporocyst residuum absent; sporozoites with a single refractile body posterior to the nucleus.

Sporulation time: Most oocysts were sporulated after 6 days at 20C.

Location in host: Unknown, recovered from feces.

Percentage infection: 156 of 629 (24.8%) snowshoe hares of central Alberta.

Remarks: *Eimeria athabascensis* most closely resembles *E. leporis* (cf. Figures 2, 15) and *E. ruficaudati* (cf. Figures 3, 18), but lacks a sporocyst residuum and a micropyle, respectively. It also resembles *E. neoleporis* of *Oryctolagus cuniculus* (Table 7), but the oocyst of *E. athabascensis* is smaller, has a micropyle, and lacks a sporocyst residuum. The specific epithet denotes the Athabasca River drainage of central Alberta (see Figure 1).

Eimeria keithi sp. n.

(Figures 6, 17)

Type host: *Lepus americanus*.

Type locality: Rochester, Alberta, Canada.

Description: Sporulated oocysts cylindrical, micropyle distinct and sunken slightly into oocyst wall, 2.4 to 5.8 (4.9 ± 0.1) wide; oocyst wall thickness about 1.5, consisting of two layers, the outer smooth and uniformly thick, being several times thicker than the inner layer; 100 sporulated oocysts 24.0 to 38.3 (32.2 ± 0.3) long, and 15.1 to 19.2 (16.9 ± 0.1) wide; polar granule absent; oocyst residuum compact, granular, 2.4 to 7.0 (4.3 ± 0.1) in diameter; sporocysts elongated spindle-shaped bodies, tapering toward one end, 12.8 to 18.0 (14.9 ± 0.1) long, and 5.8 to 7.2 (6.7 ± 0.1) wide; Stieda body present as an indistinct light refracting area at the narrow end (not shown in Figure 6, not apparent in Figure 17); sporocyst residuum absent; sporozoites with a single refractile body posterior to the nucleus.

Sporulation time: Most oocysts were sporulated after 6 days at 20C.

Location in host: Unknown, recovered from feces.

Percentage infection: 54 of 629 (8.6%) snowshoe hares of central Alberta.

Remarks: *Eimeria keithi* most closely resembles *E. ruficaudati*, *E. leporis*, and *E. athabascensis* of *Lepus* spp. and *E. honessi* of *Sylvilagus* spp. (Table 7). However, it lacks the sporocyst residuum of *E. ruficaudati*, and differs from *E. leporis* and *E. athabascensis* by possessing a distinct micropyle. It also differs from *E. leporis* by not possessing sporocyst residua. It differs from *E. honessi* in shape and size of the sporulated oocyst. The specific epithet is in honour of Dr. L. B. Keith whose extensive studies of the population dynamics of snowshoe hares are well known.

Eimeria holmesi sp. n.

(Figures 7, 14)

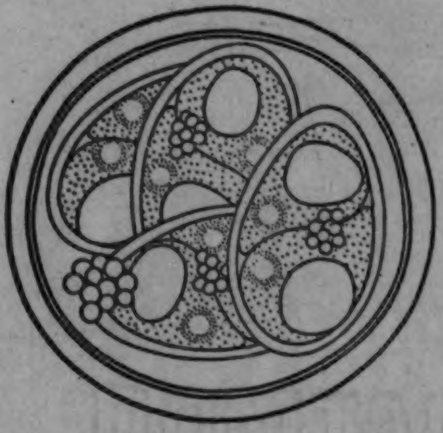
Type host: *Lepus americanus*.

Type locality: Rochester, Alberta, Canada.

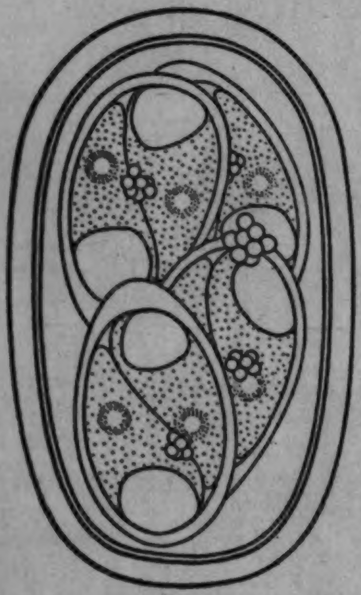
Description: Sporulated oocysts spherical or subspherical, micropyle absent; oocyst wall thickness about 1, consisting of two layers, the outer smooth and uniformly thick; 30 oocysts 13.2 to 18.6 (15.1 ± 0.3) by 12.0 to 18.6 (14.8 ± 0.3); polar granules absent; oocyst residuum small, compact, and granular; sporocysts ellipsoid, tapering toward one

Figure 7. Sporulated oocyst of *Eimeria holmesi*

Figure 8. Sporulated oocyst of *Eimeria rochesterensis*



7



8

20µm

end, 9.6 to 11.2 (10.4 ± 0.1) long, and 4.8 to 6.1 (5.2 ± 0.1) wide; Stieda body present as an indistinct light refracting area at the narrow end (not shown in Figure 7, not apparent in Figure 14); small, compact, granular sporocyst residua present; sporozoites with a single refractile body lateral or posterior to the nucleus.

Sporulation time: Most oocysts were sporulated after 6 days at 20C.

Location in host: Unknown, recovered from feces.

Percentage infection: 30 of 629 (4.8%) snowshoe hares of central Alberta.

Remarks: *Eimeria holmesi* most closely resembles both *E. hungarica* Pellérdy 1956, and *E. punjabensis* Gill and Ray 1960, but is geographically isolated from both, possesses a sporocyst residuum, and is smaller than *E. punjabensis*. The epithet is in honour of Dr. J. C. Holmes, well-known parasitologist, of the University of Alberta.

Eimeria rochesterensis sp. n.

(Figures 8, 13)

Type host: *Lepus americanus*.

Type locality: Rochester, Alberta, Canada.

Description: Sporulated oocysts cylindrical, micropyle absent; oocyst wall thickness about 1, consisting of two layers, the outer smooth, uniformly thick; 30 oocysts 20.1 to 25.8 (22.8 ± 0.3) long, and 14.4 to 15.6 (14.4 ± 0.1) wide; polar granule absent; oocyst residuum compact, small, and granular; sporocysts elongated spindle-shaped, tapering toward one end, 10.4 to 12.8 (12.2 ± 0.1) long, and 5.8 to 7.0 (6.1 ± 0.1) wide; Stieda body present as an indistinct light refracting area at the narrow end (not shown in Figure 8, not apparent in Figure 13); compact granular sporocyst residue present; sporozoites with a single refractile body posterior to the nucleus.

Sporulation time: Most oocysts were sporulated after 6 days at 20C.

Location in host: Unknown, recovered from feces.

Percentage infection: 141 of 629 (22.4%) snowshoe hares of central Alberta.

Remarks: *Eimeria rochesterensis* resembles *E. leporis, E. athabascensis, E. rowani, E. ruficaudati* and *E. keithi* of hares (*Lepus* spp.) and *E. nagpurensis* of *Oryctolagus* (Table 7). It is smaller in size than *E. leporis* and has sporocyst residua which are small and compact as opposed to the large diffuse residua of *E. leporis*. It is smaller in size than *E. athabascensis* and has a sporocyst residuum. It lacks the micropyle characteristic of *E. ruficaudati* and *E. keithi*. The presence of the oocyst residuum differentiates it from *E. nagpurensis*. It differs from *E. rowani* by having residual bodies. The specific epithet denotes the type locality.

Eimeria rowani sp. n.

(Figures 9, 12)

Type host: *Lepus americanus*.

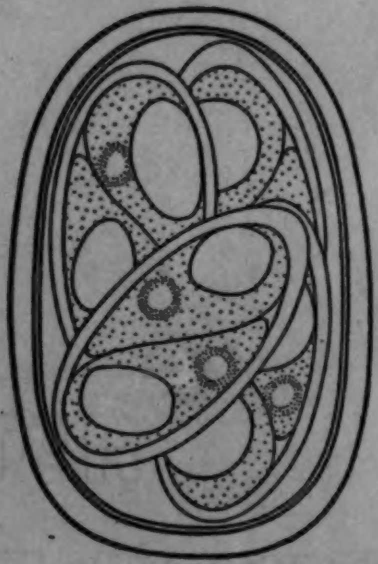
Type locality: Rochester, Alberta, Canada.

Description: Sporulated oocysts cylindrical, micropyle absent; oocyst wall thickness about 1; consisting of two layers, the outer smooth, uniformly thick; 30 oocysts 18.0 to 31.2 (22.1 ± 0.7) long, and 12.0 to 18.0 (15.8 ± 0.2) wide; polar granule absent; oocyst residuum absent; sporocysts elongated spindle-shaped bodies, tapering toward one end, 10.8 to 16.8 (12.1 ± 0.3) long, and 4.8 to 7.2 (6.0 ± 0.1) wide; Stieda body present as an indistinct light refracting area at the narrow end (not shown in Figure 9, not apparent in Figure 12); sporocyst residuum absent; sporozoites with a single refractile body posterior to the nucleus.

Sporulation time: Most oocysts were sporulated after 6 days at 20C.

Location in host: Unknown, recovered from feces.

Figure 9. Sporulated oocyst of *Eimeria rowani*

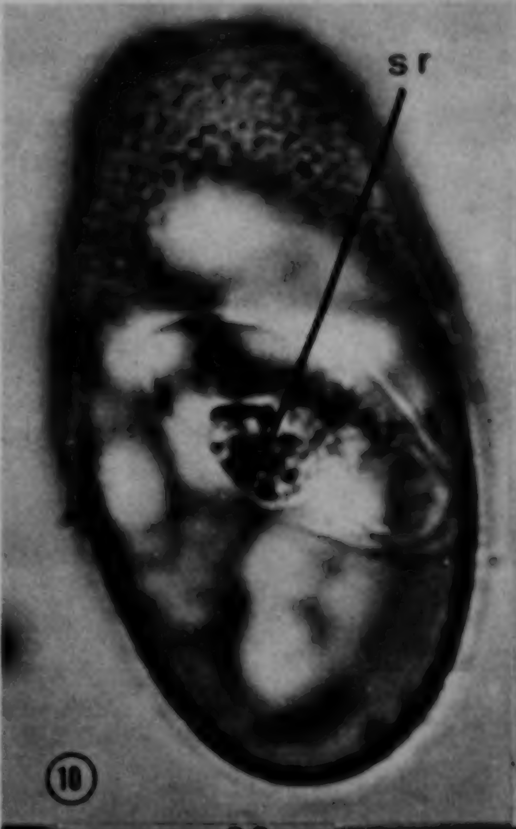


9

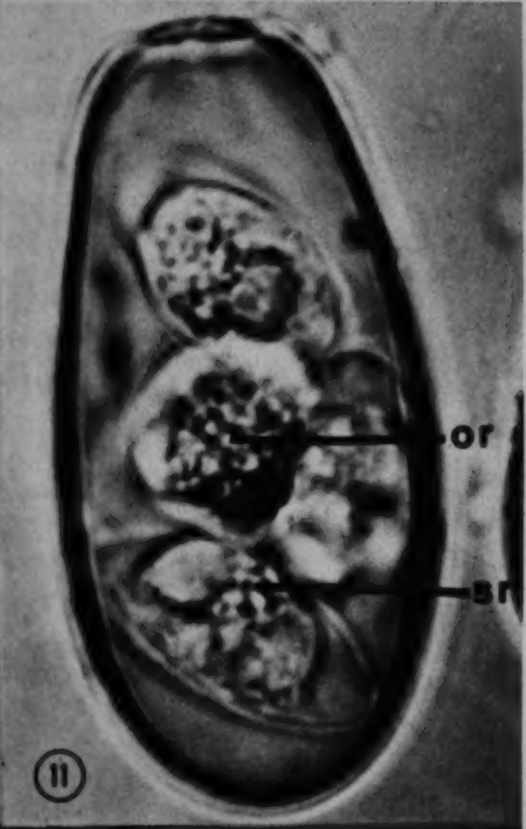
20 μm

Figures 10 - 14. Photomicrographs of sporulated oocysts of *Eimeria* spp. (or = oocyst residuum, sr = sporocyst residuum).

Figure 10. *Eimeria townsendi*
Figure 11. *Eimeria robertsoni*
Figure 12. *Eimeria rowani*
Figure 13. *Eimeria rochesterensis*
Figure 14. *Eimeria holmesi*



⑩



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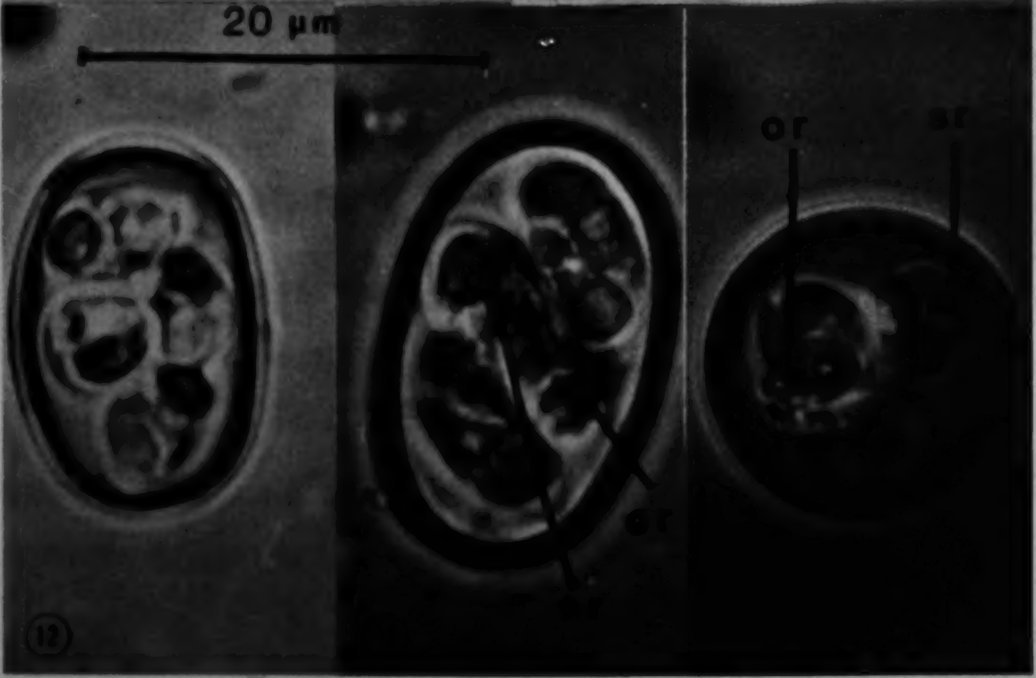
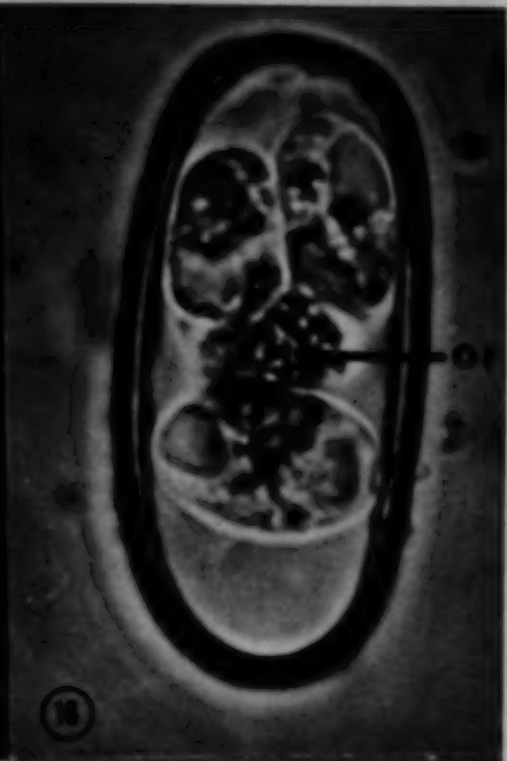
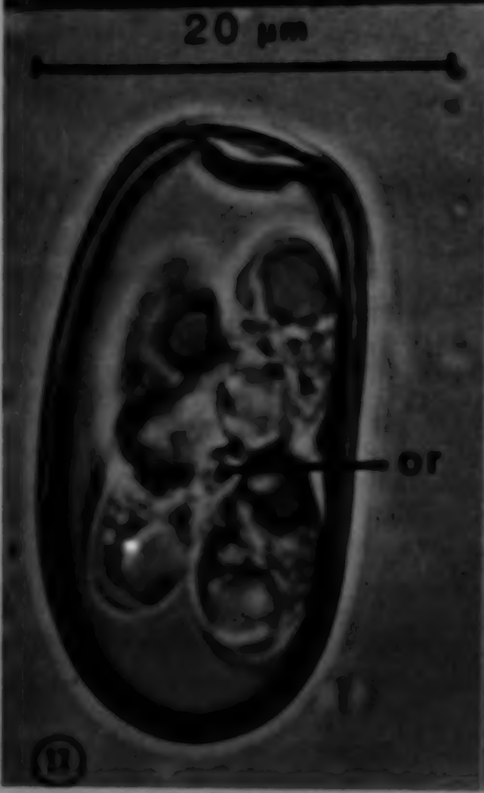

20 µm
or
sr
or
sr

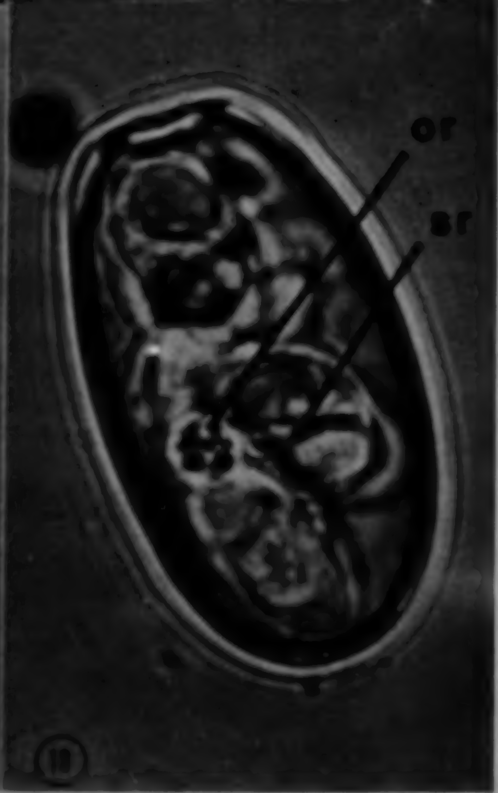
⑫

Figures 15 - 18. Photomicrographs of sporulated oocysts of *Eimeria* spp. (or = oocyst residuum, sr = sporocyst residuum).

Figure 15. *Eimeria leporis*
Figure 16. *Eimeria athabascensis*
Figure 17. *Eimeria keithi*
Figure 18. *Eimeria ruficaudati*


or
18


20 µm
or
17


or
sr
19

Percentage infection: 68 of 629 (10.8%) snowshoe hares of central Alberta.

Remarks: *Eimeria rowani* resembles *E. rochesterensis*, *E. keithi*, *E. ruficaudati*, *E. leporis* and *E. athabascensis* of hares (*Lepus* spp.) and *E. nagpurensis* of *Oryctolagus* (Table 7). It differs from *E. rochesterensis* by lacking both the oocyst and sporocyst residua. It differs from *E. keithi* and *E. ruficaudati* by not having a micropyle in addition to both types of residual bodies. It differs from *E. leporis* by the absence of sporocyst and oocyst residua, from *E. athabascensis* by the absence of the oocyst residuum, and from *E. nagpurensis* by the absence of the sporocyst residua. The specific epithet is in honour of the late Dr. William Rowan, well-known northern biologist and former Chairman of the Department of Zoology, University of Alberta.

Statistical Analysis

The species of *Eimeria* recovered from snowshoe hares in this study were placed into four groups based on size and shape of the oocysts. The groups are as follows:

Group I - *E. robertsoni* and *E. townsendi*,
Group II - *E. leporis*, *E. ruficaudati*, *E. keithi* and *E. athabascensis*,
Group III - *E. rowani* and *E. rochesterensis*, and;
Group IV - *E. holmesi*.

Within a group, species were distinguished by the presence or absence of a micropyle, oocyst residuum, and sporocyst residuum.

Groups I and IV are easily distinguished from each other and the other groups: *E. holmesi* is spherical and small (about 14 in diameter); *E. townsendi* and *E. robertsoni* are ellipsoid or ovoid and large (about 40 long). Groups II and III are similar in shape, cylindrical, and intermediate in size between Groups I and IV. Members of Group II are about 30 - 34 in length, while Group III species are about 22 in length.

There are distinct differences in morphology between and within the species groups, except for *E. leporis* from Group II and *E. rochesterensis* from Group III, which are distinguished from each other only on the basis of size. The six species in Groups II and III were subjected to a simple statistical analysis to determine if there were significant size differences which would support the designation of species based on presence or absence of the morphological characters used.

The mean, standard deviation, and standard error of the mean for oocyst width, oocyst length, sporocyst width, and sporocyst length are presented in Table 2 for all of the species observed in this study. An analysis of variance was done on the four parameters for the species in Groups II and III (Table 3). The null hypothesis, that all of the species came from the same population was rejected for all four parameters, and the alternate hypothesis, that at least some of the species means are different from each other was accepted.

The six species of Groups II and III were subjected to the New Duncan Multiple Range Test (Duncan 1955) in order to identify which means were different. Differences were found in at least one, and usually two

Table 2. Mean, standard deviation, and standard errors for oocyst length and width for the nine types of oocysts designated as species in this study

		Mean	Standard Deviation	Standard Error
Group I:				
E. robertsoni (n=100)	OL*	41.326	4.319	.432
	OW*	22.500	1.390	.139
	SL*	20.928	1.879	.188
	SW*	8.028	.631	.063
E. townsendi (n=100)	OL	39.588	2.154	.215
	OW	22.884	.840	.084
	SL	18.360	.911	.091
	SW	9.100	.581	.058
Group II:				
E. leporis (n=100)	OL	32.133	3.060	.306
	OW	15.550	.770	.077
	SL	14.440	1.457	.146
	SW	6.260	.479	.048
E. athabascensis (n=100)	OL	33.778	2.667	.267
	OW	15.600	.818	.082
	SL	15.144	1.227	.123
	SW	6.220	.474	.047
E. ruficaudati (n=100)	OL	29.938	3.043	.304
	OW	17.510	1.210	.121
	SL	15.220	1.634	.164
	SW	6.592	.630	.063
E. keithi (n=100)	OL	32.190	2.999	.299
	OW	16.902	1.042	.104
	SL	14.913	1.018	.102
	SW	6.675	.474	.047
Group III:				
E. rowani (n=30)	OL	22.040	4.054	.740
	OW	15.800	1.264	.231
	SL	12.120	1.618	.295
	SW	6.040	.384	.070
E. rochesterensis (n=30)	OL	22.830	1.545	.282
	OW	14.440	.219	.040
	SL	12.240	.686	.125
	SW	6.080	.516	.094
Group IV:				
E. holmsei (n=30)	OL	15.140	1.566	.286
	OW	14.760	1.512	.276
	SL	10.360	.725	.132
	SW	5.200	.397	.072

* OL = oocyst length; OW = oocyst width;
SL = sporocyst length; SW = sporocyst width.

Table 3. Analysis of variance of oocyst and sporocyst length and width among species group numbers II and III

	Degrees of Freedom	Sum of Squares	Mean Square	F	Significance Level
OL*					
between groups	5	5538.920	1107.784	126.272	$p < 0.001$
within groups	454	3982.940	8.773		
total	459	9521.860			
OW*					
between groups	5	396.230	79.246	84.664	$p < 0.001$
within groups	454	424.944	0.936		
total	459	821.174			
SL*					
between groups	5	430.945	86.189	47.936	$p < 0.001$
within groups	454	816.292	1.798		
total	459	1247.237			
SW*					
between groups	5	23.591	4.718	17.804	$p < 0.005$
within groups	454	120.310	0.265		
total	459	143.901			

* OL = oocyst length; OW = oocyst width; SL = sporocyst length; SW = sporocyst width

or more, of the four parameters tested for each of the possible permutations of the six species (Figure 19). Group II species differed from Group III species in at least two parameters, oocyst length and sporocyst length, for all combinations. The oocyst and sporocyst lengths of *E. leporis* from Group II and *E. rochesterensis* from Group III, two species that are morphologically similar, were significantly different ($P \leq 0.01$). Within Group II there are significant differences in at least two parameters for each combination of two species. Within Group III, *E. rochesterensis* and *E. rowani* differ significantly in oocyst width. In summary, there are significant size differences between the six species in Groups II and III as well as morphological differences which strengthens their identity as species.

Key to Species

The major features of all species of *Eimeria* reported from the genus *Lepus* are presented in Table 4. A total of 19 species of *Eimeria* from eight species of *Lepus* is recognized. Based on previously published descriptions and those in the present study, a key to the species of *Eimeria* found in *Lepus* is provided.

Key To Species Of *Eimeria*
Parasitizing Members Of The Genus *Lepus*

1. Oocysts spherical 2

    oocysts cylindrical, or ovoid to ellipsoid 5

Figure 19. Differences between oocyst and sporocyst lengths and widths of six species in Groups II and III as determined by the New Duncan Multiple Range Test. Symbols OL, OW, SL and SW indicate significant differences at $P < 0.01$. (OL = oocyst length, OW = oocyst width, SL = sporocyst length, SW = sporocyst width)

	E. athabascensis	*E. rowani*	*E. rochesterensis*	*E. ruficaudati*	*E. leporis*
E. rowani	OL SL				
E. rochesterensis	OL OW SL	OW			
E. ruficaudati	OL OW SW	OL OW SL SW	OL OW SL SW		
E. leporis	OL SL	OL SL	OL OW SL	OL OW SL SW	
E. keithi	OL OW SW	OL OW SL SW	OL OW SL SW	OL OW	OW SW

Table 4. Characters of oocysts of *Eimeria* described from the host genus *Lepus*

Species	Host	Reference	# Oocysts measured	Oocyst size[1]	Micropyle	Oocyst Residuum	Polar Body
E. americana	L. townsendii	Carvalho 1943	-	34-43x18-25 (38.1x21.0)	+	+	-
E. athabascensis	L. americanus	present study	100	24.0-38.4x13.2-18.8 (33.8x15.6)	-	+	-
E. belorussica	L. europaeus L. timidus	Litvenkova 1969	-	26-28x14-16	+	?	?
E. europaea	L. europaeus	Pellerdy 1956	-	26-34x15-20 (32x18)	+	+	-
E. holmesi	L. americanus	present study	30	13.2-18.6x12.0-18.6 (14.8x15.1)	-	+	-
E. hungarica	L. europaeus	Pellerdy 1956	-	13-15x12-14 (14x23)	-	+	-
E. keithi	L. americanus	present study	100	24.0-38.3x15.1-19.2 (32.2x16.9)	+	+	-
E. leporis	L. europaeus	Wieschulz 1923	-	26-38x13-20 (32x16)	-	+	-
	L. americanus	present study	100	26-39.6x12.8-16.8 (32.1x15.6)	-	+	-
E. punjabensis	L. ruficaudata	Gill and Ray 1960	100	20-23.8x19.5-22.5 (22.5x22)	-	+	-
E. robertsoni	L. townsendii	Carvalho 1943	-	35.7-46.8x22.8-32.8 (40.5x25.0)	+	+	-
	L. americanus	present study	100	33.6-51.6x20.4-25.2 (41.3x22.5)	+	+	-
E. rochesterensis	L. americanus	present study	30	20.1-25.8x14.4-15.6 (22.8x14.4)	-	+	-
E. rowani	L. americanus	present study	30	18.0-31.2x12.0-18.0 (22.1x15.8)	-	-	-
E. ruficaudati	L. ruficaudatum	Gill and Ray 1960	100	28-35x12-15 (31.3x17.5)	+	+	-
	L. americanus	present study	100	22.8-38.4x13.9-19.2 (29.9x17.5)	+	+	-
E. sculpta	L. arcticus	Madsen 1938	152	32-42x23-32 (36.8x28.9)	+	-	-
E. semisculpta	L. europaeus	Pellerdy 1956	-	35-45x22-27 (38x25)	+	-	-
E. septentrionalis	L. timidus	Madsen 1938	-	24-32x20-22 (27x21.6)	+	-	-
E. stefanski	L. europaeus	Pastuszko 1961a	-	59.2-68.1x32.5-37.2 (?)	+	+	-
E. stiedai	Lepus spp.	Pellerdy 1974	-	28-40.6x16-25 (37x21)	+	-	-
E. townsendi	L. europaeus	Pellerdy 1956	-	37-44x25-31 (40x28)	+	-	-
	L. americanus	present study	100	36-49x19-24 (39.6x22.9)	+	-	-

* from figures

1 measurements in micrometers, mean in parentheses

2 sporocysts residua are figured in his paper, but he states that they are absent errors present in original description

Table 4. Continued.

Species	Wall Layers	Wall Texture	Shape	Sporocyst Size^1	Stieda body	Sporocyst residuum	Geographical location
E. americana	1*	smooth	ovoid	7.0-8.0x17.1	+	-	USA (Iowa)
E. athabascensis	2	smooth	cylindrical	6.0-7.6x12.0-18.0 (6.2x15.1)	+	-	Canada (Alberta)
E. belorussica	2	?	ovoid	?	?	?	Byelorussia
E. europaea	2*	smooth	ellipsoid	9.0x6.0	+	-^{3}	Hungary
E. holmesi	2	smooth	spherical	4.8-6.1x9.6-11.2 (5.2x10.4)	+	+	Canada (Alberta)
E. hungarica	2*	smooth	spherical	?	?	-	Hungary
E. keithi	2	smooth	cylindrical	5.8-7.2x12.8-18.0 (6.7x14.9)	+	-	Canada (Alberta)
E. leporis	2*	smooth	cylindrical	6.5x12.5	?	+	Europe
	2	smooth	cylindrical	5.8-7.2x11.0-18.0 (6.3x14.4)	+	+	Canada (Alberta)
E. punjabensis	1*	smooth	spherical	8.5-12.5	+	-	India
E. robertsoni	2	smooth	ovoid	7.0x19.5-22.0	+	-	Greenland; USA (Iowa)
	2	smooth	ovoid	6.0-9.6x18.0-25.2 (8.0x20.9)	+	+	Canada (Alberta)
E. rochesterensis	2	smooth	cylindrical	5.8-7.0x10.4-12.8 (6.1x12.2)	+	+	Canada (Alberta)
E. rowani	2	smooth	cylindrical	4.8-7.2x10.8-16.8 (6.0x12.1)	+	-	Canada (Alberta)
E. ruficaudati	1	smooth	cylindrical	?	+	+	India
	2	smooth	cylindrical	5.8-8.1x11.6-18.0 (6.6x15.2)	+	+	Canada (Alberta)
E. sculpta	2*	rough	ovoid	9.0-10.0x15.0-19.0 (9.5x17.1)	+	+	Greenland
E. semisculpta	2*	rough	ellipsoid	9.0x18.0	+	+	Hungary
E. septentrionalis	2*	smooth	ellipsoid	6.0-8.0x12.0-14.0	+	-	Russia
E. stefanski	1*	smooth	ellipsoid	15.0 in length	+	-	Poland
E. stiedai	2	smooth	ovoid	10.0x18.0	+	+	Europe
E. townsendi	2	smooth	ovoid	10.0x17.0	+	+	Europe
	2	smooth	ovoid	8.4-9.6x16.8-20.4 (9.1x18.4)	+	+	Canada (Alberta)

2.	Oocysts with micropyle present	*E. septentrionalis*
	oocysts with micropyle absent	3
3.	Oocyst with well-defined oocyst residuum and sporocyst residuum	*E. holmesi*
	oocyst with well-defined oocyst residuum, but without a sporocyst residuum	4
4.	Oocysts greater than 19 in diameter	*E. punjabensis*
	oocysts less than 16 in diameter	*E. hungarica*
5.	Oocysts cylindrical	6
	oocysts ovoid or ellipsoid	11
6.	Oocysts with micropyle present	7
	oocysts with micropyle absent	8
7.	Oocyst residuum present, sporocyst residuum absent	*E. keithi*
	oocyst residuum present, sporocyst residuum present	*E. ruficaudati*
8.	Oocyst residuum present	9
	oocyst residuum absent	*E. rowani*
9.	Sporocyst residuum present	10
	sporocyst residuum absent	*E. athabascensis*
10.	Oocyst greater than 26 in length	*E. leporis*
	oocyst less than 26 in length	*E. rochesterensis*
11.	Micropyle present, oocyst residuum present	12
	micropyle present, oocyst residuum absent	15
12.	Sporocyst residua present	*E. robertsoni*
	sporocyst residua absent	13

13. Oocyst length greater than 55 . . . . *E. stefanski*

oocyst length less than 50 14

14. Oocyst length less than 50, and greater than 34, oocyst residua dispersed within the oocyst . . . . *E. americana*

oocyst length less than 34, oocyst residua compact . . . . *E. europea*

15. Oocysts found in bile ducts . . . . *E. stiedai*

oocysts not found in bile ducts 16

16. Oocyst length greater than 32 17

oocyst length less than 32 . . . . *E. belorussica*

17. Outer oocyst wall smooth . . . . *E. townsendi*

outer oocyst wall granular or pitted 18

18. Outer oocyst wall granular on micropylar half only . . . . *E. semisculpta*

outer oocyst wall granular over entire surface . . . . *E. sculpta*

A table summarizing the known *Lepus* - *Eimeria* associations is also provided (Table 5). It is likely the hosts with the highest numbers of *Eimeria*, *L. americanus*, *L. europaeus*, *L. timidus*, and *L. townsendii*, have been studied more intensively.

Host Specificity and Phylogeny

Cross-transmission experiments between three genera (*Lepus*, *Oryctolagus*, and *Sylvilagus*) of the Leporidae have been conducted using 22 species of *Eimeria* (Table 6). Success has been mixed with only six species of *Eimeria* having been transferred successfully. Five,

Table 5. A checklist of the *Eimeria* spp. reported from *Lepus* spp.

Eimeria	*Lepus* *americanus*	*arcticus*	*californicus*	*europaeus*	*ruficaudati*	*timidus*	*tolai*	*townsendi*	Total No. Hosts With *Eimeria* sp.
E. americana								+	1
E. athabascensis	+								1
E. belorussica				+		+			2
E. europaea				+					1
E. holmesi	+								1
E. hungarica				+		+			2
E. keithi	+								1
E. leporis	+	+	+*	+	+	+	+		7
E. punjabensis					+				1
E. robertsoni	+	+	+*	+	+	+		+	7
E. rochesterensis	+								1
E. rowani	+								1
E. ruficaudati	+				+				2
E. sculpta		+						+	2
E. semisculpta		+		+		+		+	4
E. septentrionalis		+				+		+	3
E. stefanski				+					1
E. stiedai			+	+				+	3
E. townsendi	+		+	+		+		+	5
Total no. *Eimeria* spp.	9	5	4	9	4	7	1	7	

* from measurements and photographs in Henry 1932

E. irresidua, E. magna, E. media, E. perforans, and *E. stiedai*, were transmitted to *Sylvilagus* from *Oryctolagus*, while two, *E. media* and *E. neoleporis*, were established in *Oryctolagus* from *Sylvilagus*. There are no reports of successful transmissions of *Eimeria* between *Lepus* and *Sylvilagus*. There is only one reliable report of successful transmission of a species of *Eimeria, E. stiedai*, from *Oryctolagus* to *Lepus* (Donciu *et al*. 1968; see Table 6).

The reports of transmission of *E. stiedai, E. media*, and *E. magna* between *Lepus* and *Oryctolagus* in an abstract by Burgaz (1973) must be viewed with caution. *E. media* is an unlikely parasite of the genus *Lepus* (Levine and Ivens 1972), and the presence of oocysts of *E. stiedai*, which has a prepatent period of 16 days (Pellérdy 1974), in animals 11 days post inoculation must be interpreted as due to prior infection, or a misidentification. Burgaz (1973) did not give particulars relating to identification of species, nor did she give the details of procedures followed to ensure that animals were not infected prior to exposure or did not become infected during the course of the experiment. Since the descriptions of *E. media, E. magna, E. robertsoni*, and *E. europea* are similar (the first two are known from *Oryctolagus* and *Sylvilagus*, and the last two from *Lepus*), it is possible that Burgaz (1973) may have misidentified her material.

In summary, six species (15 attempts with 13 species) of *Eimeria* have been transmitted between the genus *Sylvilagus* and the genus *Oryctolagus*, no species (7 attempts with 7 species) have been transmitted from *Lepus* to *Sylvilagus*, and one (28 attempts with 17 species) has been

Table 6. Summary of cross transmission experiments with *Eimeria* spp. of the Order Lagomorpha

Species of *Eimeria*	Transmission (sporulated oocysts)			
	Donor	Recipient	Result	Source
E. americana	4	5	-	Carvalho 1943
	4	8	-	Carvalho 1943
E. environ	8	5	-	Carvalho 1943
E. europaea	2	5	-	Pellerdy 1956
	2	5	-	Pastuszko 1961b
E. exiqua	5	2	-	Pellerdy 1956
E. honessi	8	5	-	Carvalho 1943
E. hungarica	2	5	-	Pellerdy 1956
	4	8	-	Carvalho 1943
	3	5	-	Burgaz 1973
	2	5	-	Pastuszko 1961b
E. irresidua	5	8	+	Carvalho 1943
	5	2	-	Pellerdy 1956
E. leporis	2	5	-	Nieschulz 1923
	3	5	-	Burgaz 1973
	2	5	-	Pellerdy 1956
	2	5	-	Pastuszko 1961b
E. magna	5	8	+	Becker 1933
	5	8	+	Carvalho 1943
	5	3	+*	Burgaz 1973
	5	2	-	Pellerdy 1956
E. maior	8	5	-	Carvalho 1943
E. minima	8	5	-	Carvalho 1943
E. media	8	5	+	Carvalho 1943
	5	8	+	Carvalho 1943
	3	5	+*	Burgaz 1973
	5	2	-	Pellerdy 1956
E. neoleporis	8	5	+	Carvalho 1943
E. paulistana	7	5	-	daFonesca 1933
E. perforans	5	8	+	Carvalho 1943
	5	2	-	Pellerdy 1956
	5	3	-	Burgaz 1973
E. pintoensis	7	5	-	daFonesca 1932
E. piriformis	5	3	-	Burgaz 1973
	5	2	-	Pellerdy 1956
E. robertsoni	4	5	-	Carvalho 1943
	4	8	-	Carvalho 1943
	2	5	-	Pellerdy 1956
	3	5	-	Burgaz 1973
	1	5	-	¢
	2	5	-	Pastuszko 1961b
E. sculpta	4	5	-	Carvalho 1943
	4	8	-	Carvalho 1943

Table 6 (continued)

Species of *Eimeria*	Transmission (sporulated oocysts)			
	Donor	Recipient	Result	Source
E. semisculpta	4	5	−	Carvalho 1943
	4	8	−	Carvalho 1943
	2	5	−	Pellerdy 1956
	3	5	−	Burgaz 1973
	2	5	−	Pastuszko 1961b
E. septentrionalis	4	5	−	Carvalho 1943
	4	8	−	Carvalho 1943
E. stefanskii	2	5	−	Pastuszko 1961b
E. stiedai	5	6	+	Jankiewcz 1941
	5	2	+	Donciu *et al.* 1968
	5	3	+*	Burgaz 1973
E. townsendi	4	5	−	Carvalho 1943
	4	8	−	Carvalho 1943
	2	5	−	Pellerdy 1956
	2	5	−	Pastuszko 1961b

★ Reports by Burgaz are not verified - see text p.
♦ Samoil and Samuel unpub. M.Sc. - see App. I
1 = *Lepus americanus*
2 = *Lepus europaeus*
3 = *Lepus timidus*
4 = *Lepus townsendii*
5 = *Oryctolagus cuniculus*
6 = *Sylvilagus audubonii*
7 = *Sylvilagus brasiliensis*
8 = *Sylvilagus floridanus*

transmitted between *Oryctolagus* and *Lepus*. All of this suggests that, based on results of cross-transmission studies, the genera *Oryctolagus* and *Sylvilagus* are more closely related to each other than they are to the genus *Lepus*.

In an attempt to determine whether there is any structural character or group of characters which might be used to differentiate the oocysts of *Eimeria* between host genera in the Leporidae, the known structural characters of the oocysts of *Sylvilagus* and *Oryctolagus* (Table 7) were compared with those of *Lepus* (Table 4). The results are summarized in Table 8; there are no clear cut characters of oocysts of *Eimeria* that connect one genus more closely with another. These characters were then summarized for the Leporidae (Table 8) and compared with the eimerian features of the Ochotonidae, Rodentia, and Artiodactyla (Table 9) in order to determine whether one can use structural characters of the *Eimeria* to differentiate host taxa.

The *Eimeria* of the Leporidae generally have a micropyle (76%), never have a micropylar cap, and usually lack a polar body (98%). They may (56%) have an oocyst residuum, usually have a sporocyst Stieda body (94%) and a sporocyst residuum (65%). In contrast, the *Eimeria* of the Ochotonidae may have a micropyle (57%), may have a polar body (50%), and, like the Leporidae, never have a micropylar cap. They usually lack an oocyst residuum (79%), have a sporocyst residuum (79%), and a Stieda body has been observed in all the species in which it has been looked for (Hobbs and Samuel 1974). Thus, the *Eimeria* of the Ochotonidae differ from those of the Leporidae in the frequency of occurrence of a micropyle,

Table 7. Characters of the species of *Eimeria* from the Family Leporidae excluding the genus *Lepus* (compiled from Pellerdy, 1974)

Species	Type Host*	Oocyst Size ¢	Micropyle	Oocyst Residuum	Polar Body	Shape	Wall Layers - Texture	Sporocyst Residua	Stieda Body	Geographical Location
E. audubonii	*S. audubonii*	21.2x17.1	+	-	-	ovoid	2 - smooth	-	+	U.S.A.
E. environ	*S. nuttallii*	25.7x18.5	+	-	-	ovoid	2 - smooth	-	+	U.S.A.
E. honessi	*S. floridanus*	28.2x19.6	+	+	-	ovoid	2 - smooth	-	+	U.S.A.
E. maior	*S. nutallii*	48.4x29.5	+	+	-	ovoid	2 - rough	+	+	U.S.A.
E. minima	*S. floridanus*	13.4x10.8	-	-	-	ovoid	2 - smooth	+	+	U.S.A.
E. neoirresidua	*S. audubonii*	25.7x17.9	+	-	-	ovoid	2 - smooth	+	+	U.S.A.
E. neoleporis	*S. floridanus*	38.8x19.8	+	-	-	cylindrical	? - smooth	+	+	U.S.A.
E. paulistana	*S. brasiliensis*	43.0x24.0	+	-	-	ellipsoid	3 - smooth	+	-	Brazil
E. pintoensis	*S. brasiliensis*	23.5x15.5	+	-	-	ovoid	1 - smooth	+	?	Brazil
E. poudrei	*S. audubonii*	26.0x18.1	+	+	-	ovoid	2 - smooth	+	+	U.S.A.
E. sylvilagi	*S. brasiliensis*	29.0x17.5	+	-	-	ovoid	2 - smooth	+	+	Brazil, U.S.A.
E. coecicola	*O. cuniculus*	25-40x14-21	+	+	-	ovoid	? - smooth	+	?	USSR, Hungary
E. exigua	*O. cuniculus*	14.5x12.7	-	-	-	spherical	? - smooth	?	?	Europe
E. intestinalis	*O. cuniculus*	27.0x18.0	+	+	-	pear shaped	? - smooth	?	?	USSR, Hungary
E. irresidua	*O. cuniculus*	38.3x25.6	+	-	-	ovoid	? - smooth	+	?	U.S.A.
E. magna	*O. cuniculus*	35.0x24.0	+	+	-	ovoid	2 - smooth	+	+	France
E. matsubayashii	*O. cuniculus*	25.0x18.0	+	+	-	ovoid	? - smooth	?	?	?
E. media	*O. cuniculus*	31.2x18.5	+	+	-	ovoid	? - smooth	+	-	U.S.A.
E. nagpurensis	*O. cuniculus*	23.0x13.0	-	-	-	cylindrical	? - smooth	+	?	India
E. oryctolagi	*O. cuniculus*	28-46x14-23	+	+	-	ovoid	2 - smooth	+	+	India
E. perforans	*O. cuniculus*	22.7x14.2	+	+	+	ellipsoid	? - smooth	+	+	Europe
E. piriformis	*O. cuniculus*	29.0x18.0	+	-	?	piriform	2 - smooth	+	?	Hungary
E. stiedai	*O. cuniculus*	37.0x21.0	+	-	-	ovoid	2 - smooth	+	+	Europe

* S = *Sylvilagus*, O = *Oryctolagus*
¢ mean values of oocyst length and oocyst width, in micrometers

Table 8. Summary of characters of Eimeria spp. of the genera of Lagomorpha

	Host Genera			
	Ochotona	Lepus	Sylvilagus	Oryctolagus
No. eimerians	14	19	11	13
No. with micropyle	8	11	10	11
No. with oocyst residuum	3	12	4	8
No. with polar body	7	0	0	1
No. with stieda body	8	19	9	5
No. with sporocyst residuum	11	11	8	10

Table 9. Summary of various characters of *Eimeria* spp. of selected host taxa

Host taxa	Micropyle Present	Micropyle Cap Absent	Polar Body Absent	Oocyst Residuum Present	Sporocyst Residuum Present	Stieda Body Present
Order Lagomorpha	71%	100%	86%	47%	69%	95%
Family Leporidae	76%	100%	98%	56%	65%	94%
Family Ochotonidae[1]	57%	100%	50%	21%	79%	100%
Order Artiodactyla[2]	72%	77%	47%	10%	84%	73%
Family Cervidae[2]	68%	96%	70%	9%	75%	64%
Family Bovidae[2]	74%	74%	40%	11%	87%	78%
Order Rodentia[3]	18%	99%	43%	35%	89%	60%

[1] Hobbs and Samuel 1974

[2] Levine and Ivens 1970, Table 11

[3] Levine and Ivens 1965, Table 31

polar body, and oocyst residuum.

All of the species of coccidia found in the Leporidae belong to the genus *Eimeria*, while two species of *Isospora* as well as 14 species of *Eimeria* (Hobbs and Samuel 1974) have been found in the Ochotonidae.

The *Eimeria* of the Artiodactyla tend to have a micropyle (72%), lack a micropylar cap (77%), and a polar body may be present (53%). They usually lack an oocyst residuum (90%), but have a sporocyst residuum (84%) and a sporocyst Stieda body (73%). There is some variation in this pattern at the family level. The *Eimeria* of the Cervidae usually lack an oocyst polar body (70%) and a micropylar cap (96%), whereas the Bovidae tend to have a polar body (60%) (Table 9). The general absence of an oocyst residuum would appear to be a major differentiating character spearating the *Eimeria* of leporids and artiodactyls. Based on the characters analysed, there is a great deal of similarity in the coccidia of the ochotonids and the artiodactyls.

The *Eimeria* of the Rodentia have characters markedly different from the other groups discussed previously. They usually lack a micropyle (82%) and a micropylar cap (99%), and have an oocyst polar body (57%). They usually lack an oocyst residuum (65%), may have a sporocyst Stieda body (60%), and usually have a sporocyst residuum (89%) (Table 9).

In summary, there is much variability in presence or absence of major structural characters of oocysts of major taxa, be they genera of leporids or orders of mammals. There are no clear cut relationships of the genera within the leporids or of all genera within the lagomorphs based only on presence or absence of major characters of oocysts. The

Eimeria spp. of lagomorphs can be differentiated generally from those of rodents because they possess a micropyle and lack a polar body. They can be distinguished from *Eimeria* spp. of the artiodactyls by the absence of a micropylar cap and polar body.

Discussion

The snowshoe hare, *Lepus americanus*, is one of 45 species of the Lagomorpha and one of three species of the Leporidae in Alberta (Soper 1964). It is sympatric with species of the two families in the order: *Ochotona princeps* (Ochotonidae) and *Lepus townsendii* (Leporidae), but does not overlap with *Sylvilagus nuttallii* (Leporidae) (Soper 1964).

The family Leporidae contains eight genera (Layne 1967): *Pentalagus* on Ryukyu Island, *Caprolagus* and *Nesolagus* in Asia, *Pronolagus* in South Africa, *Romerolagus* in Central Mexico, *Sylvilagus* in North and South America, *Oryctolagus* in Europe, North Africa, Australia,* and New Zealand, and *Lepus* in Europe, Asia, Africa, and North America. The most successful genera are *Lepus*, *Oryctolagus*, and *Sylvilagus*.

Phylogenies of the leporids and lagomorphs have been presented by Hibbard (1963) and Gureev (1964) respectively. Hibbard's (1963) scheme was based on tooth developmental patterns; his analysis suggested that *Lepus*, *Oryctolagus*, *Caprolagus*, and *Sylvilagus* were in the same group, all derived from a pro-*Sylvilagus* stock (figure 3 of Hibbard 1963). In addition, *Lepus* and *Oryctolagus* were considered to be more closely related to each other than to *Sylvilagus*.

---

*introduced

Gureev's (1964) scheme was based on morphological, physiological, and ecological details recorded for the lagomorphs. The family Leporidae was divided into a number of subfamilies, one of which, the Leporinae, contained five tribes. All the recent genera of the leporids were placed in the tribes Pentalgini, Oryctolagini (*Sylvilagus* and *Oryctolagus*), and Leporini (*Lepus*).

An attempt was made to analyze data of this study and that published previously on lagomorph coccidia and life history in order to confirm or refute the suggested phylogenies. Most evidence seems to support the scheme of Gureev. Results of cross-transmission studies (Table 6) suggest that *Sylvilagus* and *Oryctolagus* are more closely related to each other than to *Lepus*. Other biological evidence is less clear but tends to support this conclusion as well. For example, Matthey (1973) and Hsu and Benirschke (1967, 1970, 1971) reported that the diploid chromosome number (2N) for nine species of *Lepus* was 48 and for the one species of *Oryctolagus*, 44. Three species of *Sylvilagus* have a diploid number of 42; one species, 48; and one, 52. Unfortunately, insufficient information was given to determine whether or not the fundamental number (FN) of *Sylvilagus* is constant. All species of *Sylvilagus* used in cross-transmission experiments of coccidia for which the chromosome number is known have a 2N of 42.

Matthey (1973) presented evidence to suggest that the direction of evolution is toward smaller 2N numbers. According to Gureev's scheme, *Lepus*, which has a higher chromosome number than *Sylvilagus* and *Oryctolagus*, is in a tribe which arose in the late miocene, while the other two belong to a tribe which arose in the early pliocene.

There is also evidence from the life historv of members of these three genera which suggest that *Oryctolagus* and *Sylvilagus* are more closely related to each other than to *Lepus*. Members of the genus *Oryctolagus* excavate extensive communal burrow systems (Layne 1967). One species of *Sylvilagus, S. idahoensis*, excavates its own burrows (Hall and Kelson 1959), while the majority of the species utilize abandoned burrows of other animals (Hall and Kelson 1959). Members of the genus *Lepus* do not utilize burrows but will hide or rest in brush piles, dense grass, or woody areas (Banfield 1967) or use "forms", which are depressions in the soil or vegetation made by the animal (Layne 1967).

The young of *Oryctolagus* and *Sylvilagus* are precocial and born in a fur-lined nest prepared by the female (Lord 1963), while the young of *Lepus* are altricial and born on the ground (Hall and Kelson 1959, Layne 1967, Rongstad and Tester 1971). The gestation period for *Sylvilagus* is 26 to 30 days (average 28) *Oryctolagus*, 28 to 35 days (average 30) and *Lepus*, 30 to 47 days (average 38) (Layne 1967). These data, along with results of cross-transmission studies with coccidia, suggest that *Sylvilagus* and *Oryctolagus* are more closely related to each other than they are to *Lepus*; this supports the scheme of relationships proposed by Gureev (1964). Structural components of the oocysts of *Eimeria* from these host genera do not strengthen or weaken Gureev's scheme.

If host-parasite information is useful as an indicator of phylogenetic relationships within a particular group of closely related animals such as the leporids, perhaps parasitological evidence can be used to show relationships between higher taxa. Historically, the lagomorphs were considered to be closely related to rodents and were placed within the suborder Duplicidentata in the order Rodentia, but Gidley (1912) placed the Duplicidentata in an independent order, the Lagomorpha. He suggested that the two criteria by which the lagomorphs had been linked to the Rodentia, similar development of scalpriform incisors and similarities in morphology of the brain and reproductive system, were outweighed by the anatomical differences seen in the skull and feet. Gidley, when speculating on the origin of the lagomorphs, pointed out a number of characters in which they paralleled the higher ungulates: the broad palate, the manner of chewing on one side at a time with a lateral motion of the jaw, upper cheek-teeth wider than the lower ones, and similar modifications of the limbs and feet. Gidley suggested that:

> "These characters, while perhaps in no way denoting relationships to the higher ungulates, nevertheless indicate an advance in general development beyond the Rodentia which mark the latter as the more primitive order."

Moody *et al.* (1949) suggested that lagomorphs were serologically more similar to artiodactyls than rodents, on the basis of serological tests.

An examination of the structural characteristics of *Eimeria* from rodents, artiodactyls, and lagomorphs does not reveal any clear cut relationships. The *Eimeria* of the Ochotonidae appear to be more similar to those of rodents and artiodactyls than to those of the Leporidae. The eimerians of the Leporidae show similarities with the *Eimeria* of both the rodents and artiodactyls. Thus, while host-parasite relationships of coccidia may aid in sorting out relationships within a family, they do not seem to be valuable in indicating affinities to other higher taxa.

V. ECOLOGY

Introduction

The host-parasite relationship as defined by Sprent (1963) (see page 3 of this thesis) is influenced by both the external and internal environment. According to Sprent, the external environment is "the arena where the struggle for survival is enacted", and the internal environment is "the arena where the struggle between the host and parasite is enacted".

The components of the external environment~~,~~ which may play a role in the survival of coccidian oocysts have largely been ignored. However, Kheysin (1967) reviewed two important components, temperature and humidity, and indicated that most oocysts sporulate within a range of 10 to 30C, and that the rate of sporogony increase or decrease with parallel changes in temperature. Becker and Crouch (1931) found that *E. magna* and *E. perforans* of *O. cuniculus* only sporulated between 10 and 36C, and that exposure for short periods of time (10 minutes) to temperatures above 50C resulted in immediate death of the oocysts.

Although no oocysts of any species of *Eimeria* are known to sporulate at temperatures less than 10C (Kheysin 1967), they have been shown to remain viable for long periods of time at low temperatures. Hagan (1958), for example, found that oocysts of *E. stiedai*, stored for 3.5 to 4.5 years at 5C in physiological saline solution, were capable of sporulating at room temperature. However, there was a critical low temperature; all oocysts of the same species (*E. stiedai*) were killed quickly at -10 to -15C. Kheysin (1967) cited other studies which indicated that temperatures below OC were detrimental to oocyst survival.

The highest rates of oocyst survival occur under conditions of saturated humidity (Kheysin 1967), which is also related to temperature (Strahler 1965). The summary of Kheysin's review (1967) on the combined effects of moisture and temperature on oocyst survival and sporulation is that, under conditions of less than 100% relative humidity, the sporulation times of oocysts increase and the viability of sporulated oocysts decreases.

There are a number of components of the internal environment which may affect coccidian infections. The most important ones are the combined effects of stress and immunity (see Esch *et al.* 1975, and Sprent 1963 for a discussion of these factors in relation to all host-parasite relationships). Natural or acquired immunity may result in a decreased oocyst output and a shorter patent period (Rose 1973). Stressors such as temperature, diet, and host density may affect the host directly or indirectly by acting on the immune system through increased adrenocortical activity (Esch *et al.* 1975). The resulting production of corticosteroids may act to depress or abolish existing immunity to coccidian infections.

In summary, there is likely a complex interaction of factors from the external and internal environments which may affect coccidian infections in the snowshoe hare. In the present study, selected factors were examined that may directly or indirectly influence populations of eimerians in hares. Seasonal patterns of prevalence and intensity and relationships between age and sex of the host and oocyst output may reflect changes in various stressor inputs. The major overall objective was to clarify the contributions of these factors to the epizootiology of coccidian infections. Detailed rates of the acquisition of coccidia by neonatal snowshoe hares were also collected in order to evaluate the importance of coccidiosis as a factor in juvenile mortality and the cyclic nature of the host.

Results

The prevalence and mean intensity of the 9 species of *Eimeria* observed in the feces of 629 snowshoe hares are given in Table 10. Most (78%) of the animals were infected with one or more species of *Eimeria*. Generally, the most and least prevalent species were also the most and least abundant (or numerous), respectively. There were 111 animals (17.6%) infected with only one species, 142 (22.6%) with two, 114 (18.3%) with three, 81 (12.9%) with four, 31 (4.9%) with five, 12 (1.9%) with six, and 1 (0.2%) with seven species. There were no animals infected with more than seven species.

Age

Several aspects of the "internal environment" were examined. The relationship between age and oocyst output was used as a possible measure of the effectiveness of the host's immune response to species of *Eimeria*. Linear regressions of $\log_{10}$ oocyst counts on age in days were as follows: *E. ruficaudati* $Y = 3.20 - 0.0021X$, $r = -0.42$, $P < 0.001$); *E. keithi* ($Y = 3.21 - 0.0021X$, $r = -0.38$, $P < 0.002$); *E. robertsoni* ($Y = 3.08 - 0.00081X$, $r = -0.24$, $P < 0.001$); *E. townsendi* ($Y = 3.07 - 0.00073X$, $r = -0.22$, $P < 0.001$); *E. leporis* ($Y = 3.09 - 0.00088X$,

Table 10. The prevalence and mean intensity of *Eimeria* spp. detected in 629 snowshoe hares from central Alberta, 1971-1972

Species of *Eimeria*	No. of Hares Infected	Per Cent Infected	Mean Intensity/Gram Feces ±1 Standard Deviation
athabascensis	156	24.8	593 ± 8.4
holmesi	30	4.8	185 ± 7.0
keithi	54	8.6	584 ± 8.2
leporis	223	35.5	708 ± 8.2
robertsoni	208	33.1	708 ± 9.1
rochesterensis	141	22.4	304 ± 6.0
rowani	68	10.8	113 ± 5.8
ruficaudati	101	16.1	602 ± 8.8
townsendi	314	49.9	716 ± 8.7

$r = -0.31$, $P < 0.001$); ***E. athabascensis*** ($Y = 2.98 - 0.00079X$, $r = -0.24$, $P < 0.001$); ***E. rochesterensis*** ($Y = 2.68 - 0.00082X$, $r = -0.27$, $P < 0.001$); ***E. rowani*** ($Y = 2.1500 - 0.0003X$, $r = 0.13$, $P > 0.1$); and ***E. holmesi*** ($Y = 2.4506 - 0.0005X$, $r = -0.16$, $P > 0.2$).

Season and Sex

The sex of the animal may influence the "internal environment" at certain times of the year. During the breeding season there are numerous physiological changes which may alter the internal environment of males and females in different ways. To examine the effect of sex of the animal on oocyst output, an analysis of variance was done, correcting for host age (Table 11). Age, as was shown before, was the most important factor for seven species. The sex of the animal did not affect oocyst output significantly for any species. As a consequence, all subsequent analyses will consider both sexes together.

The analysis of covariance also showed that season effects were important for five species, four of which (***E. leporis, E. athabascensis, E. robertsoni***, and ***E. townsendi***) also had significant age effects. The fifth species, ***E. rowani***, showed no age relationship to oocyst output.

Because of the significant effects of host age on oocyst output, the effects of seasonal changes were examined within three age classes. Animals were classed as either juvenile (less than one year old), yearling (one to two years old), or adult (over two years old) on the basis of their lens weights.

The mean number of species (species richness) per season for the

Table 11. Summary of results of an analysis of covariance with oocyst output as the dependent variable; season and sex as the independent variables and age (in days) as the covariate

Species of *Eimeria*	Covariate Age	Main Effects Season	Sex	2 - way Interaction Season - Sex
athabascensis	11.650(0.001)*	6.273(0.001)	1.217(NS)	0.243(NS)
holmsei	0.685(NS) ¢	0.664(NS)	0.019(NS)	1.098(NS)
keithi	8.961(0.005)	1.267(NS)	0.174(NS)	1.296(NS)
leporis	27.655(0.001)	5.883(0.001)	0.101(NS)	1.736(NS)
robertsoni	14.750(0.001)	4.164(0.001)	0.004(NS)	0.930(NS)
rochesterensis	11.342(0.001)	2.684(NS)	0.020(NS)	0.225(NS)
rowani	1.244(NS)	2.396(0.05)	0.332(NS)	1.835(NS)
ruficaudati	20.093(0.001)	0.780(NS)	1.093(NS)	0.672(NS)
townsendi	17.226(0.001)	4.005(0.001)	1.821(NS)	1.838(NS)

* F ratio with level of significance in parentheses

¢ NS - not significant

three different age classes is plotted in Figure 20. Species richness peaked for all age classes in the summer and declined through fall, winter, and spring. The peak for juveniles, which were born from May to July, came in the late summer and early fall, whereas adults and yearlings peaked in the early summer. There was more variability in data on prevalence and mean intensity for each species plotted for each host age class over time (Figures 21 and 22). However, the mean intensity in juveniles, as expected from previous analysis, was often higher than in yearlings and adults, particularly for abundant species in late summer. Although total oocyst output (Figure 22) paralleled species richness, there were no evident trends for individual species.

Rate of Acquisition and Host Density

The rate of acquisition of *Eimeria* spp. by neonatal hares as revealed by the presence and output of oocysts in the feces is summarized in Tables 12 and 13. All nine species were recovered from feces of hares approximately one week old, although intensities and prevalences varied.

The cyclic nature of hare populations in central Alberta has been repetitive and relatively invariable (Keith 1974). During the study, hares were in a steep decline phase, having peaked in the fall of 1970 and then declined drastically to the fall of 1972 (1,120 hares per km^2 in November 1970 to 150 per km^2 in 1972) (Keith 1974). An examination of the seasonal prevalence and intensity data for the species of *Eimeria* (Figures 21 and 22) observed in this study fail to supply any evidence that coccidian infections are affected by host densities, although it

Figure 20. Seasonal patterns of species richness (mean number of species per host) in the three age classes, juvenile (—▪—), yearling (--⊙--), and adult (—*—), of snowshoe hares. Collection periods are:

1 = May 1 - May 15, 1971
2 = May 16 - July 15, 1971
3 = July 16 - August 31, 1971
4 = September 1 - October 31, 1971
5 = November 1, 1971 - March 31, 1972
6 = April 1 - May 15, 1972
7 = May 16 - July 15, 1972
8 = July 16 - August 31, 1972

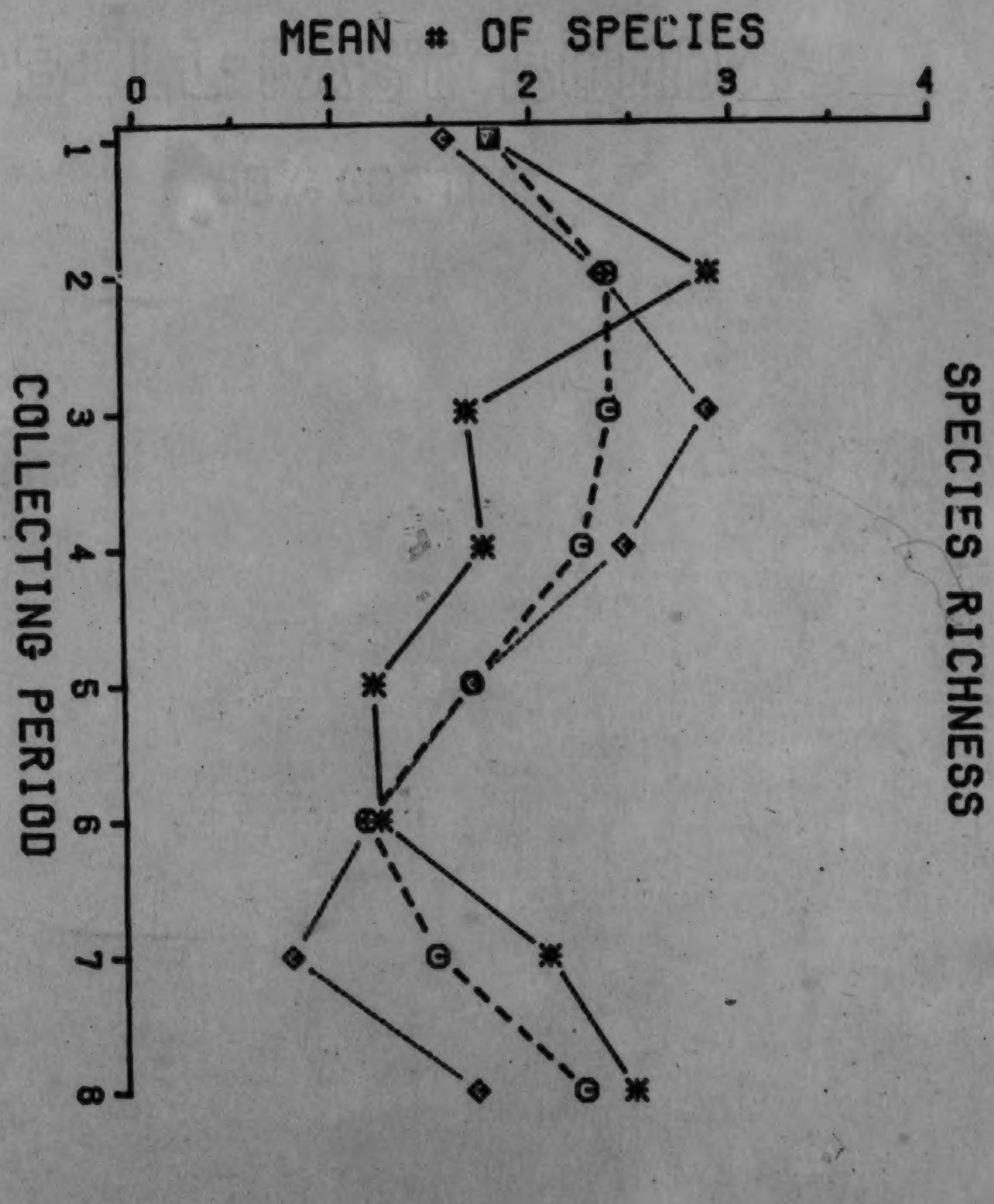
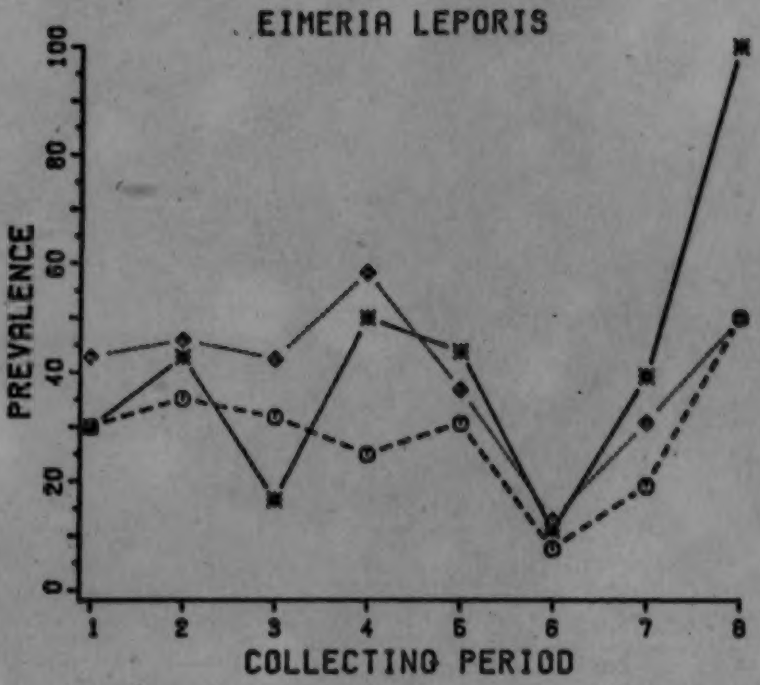

SPECIES RICHNESS
MEAN # OF SPECIES
0
1
2
3
4
COLLECTING PERIOD
1
2
3
4
5
6
7
8

Figure 21. Seasonal patterns of per cent prevalence of *Eimeria* spp. in the three age classes, juvenile (—■—), yearling (--⊙--), and adult (—✱—), of snowshoe hares. Collection periods are as listed in Figure 20.


EIMERIA LEPORIS
PREVALENCE
0
20
40
60
80
100
1
2
3
4
5
6
7
8
COLLECTING PERIOD

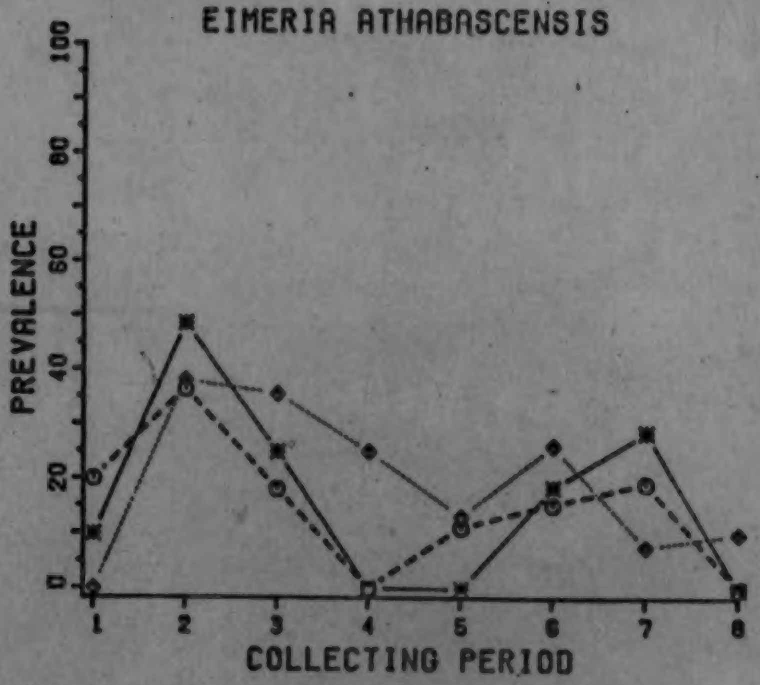
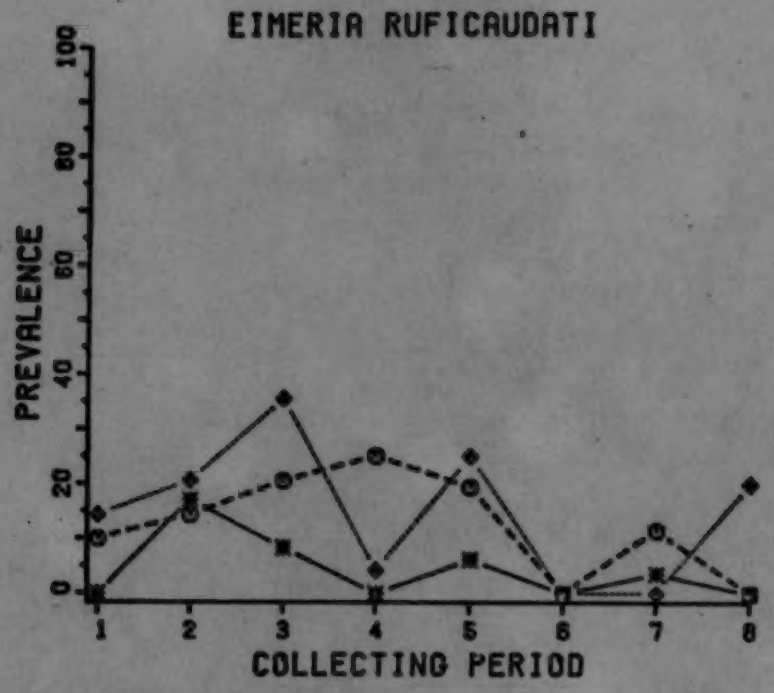
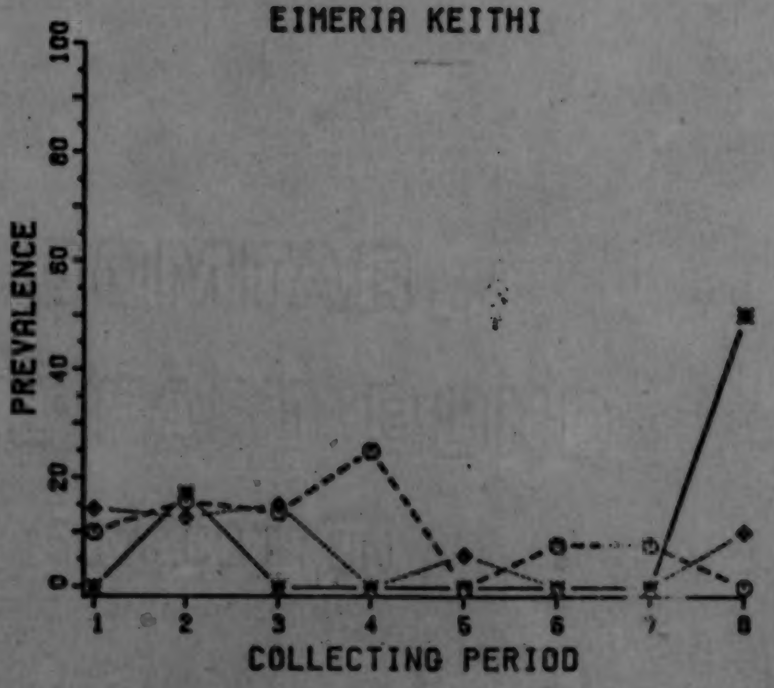

EIMERIA ATHABASCENSIS
PREVALENCE
0
20
40
60
80
100
1
2
3
4
5
6
7
8
COLLECTING PERIOD

Figure 21. Continued.

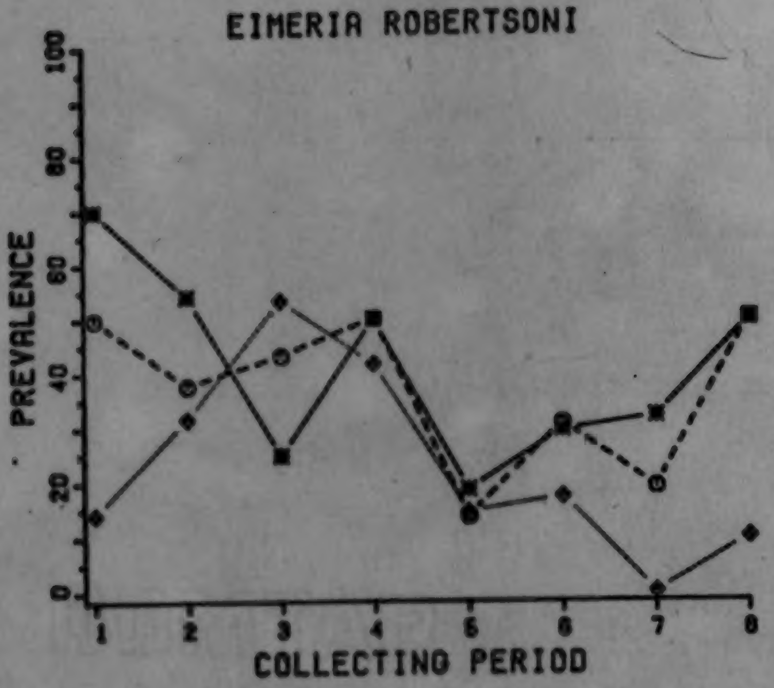


EIMERIA RUFICAUDATI



EIMERIA KEITHI

Figure 21. Continued.


EIMERIA ROBERTSONI
PREVALENCE
0
20
40
60
80
100
1
2
3
4
5
6
7
8
COLLECTING PERIOD

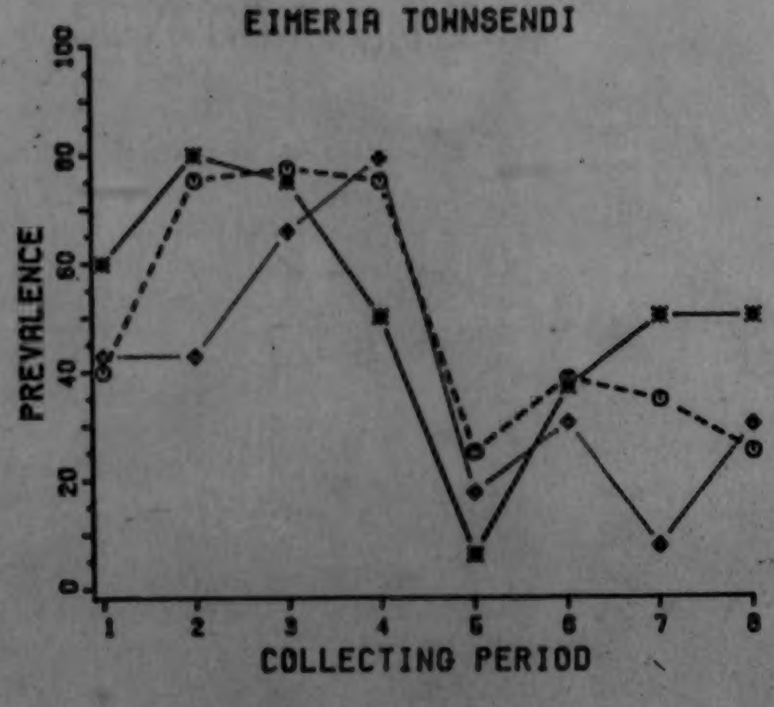
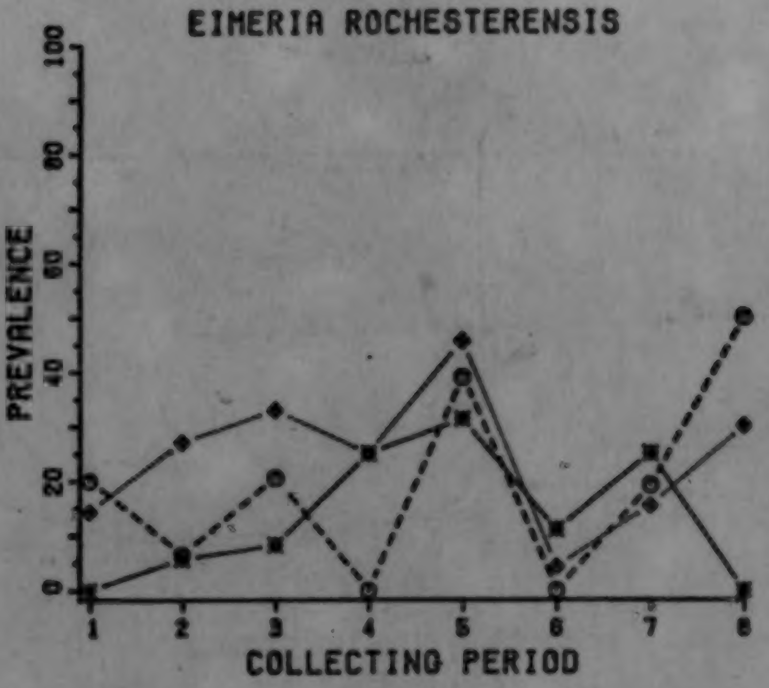

EIMERIA TOWNSENDI
PREVALENCE
0
20
40
60
80
100
1
2
3
4
5
6
7
8
COLLECTING PERIOD

Figure 21. Continued.


EIMERIA ROCHESTERENSIS
PREVALENCE
0
20
40
60
80
100
1
2
3
4
5
6
7
8
COLLECTING PERIOD

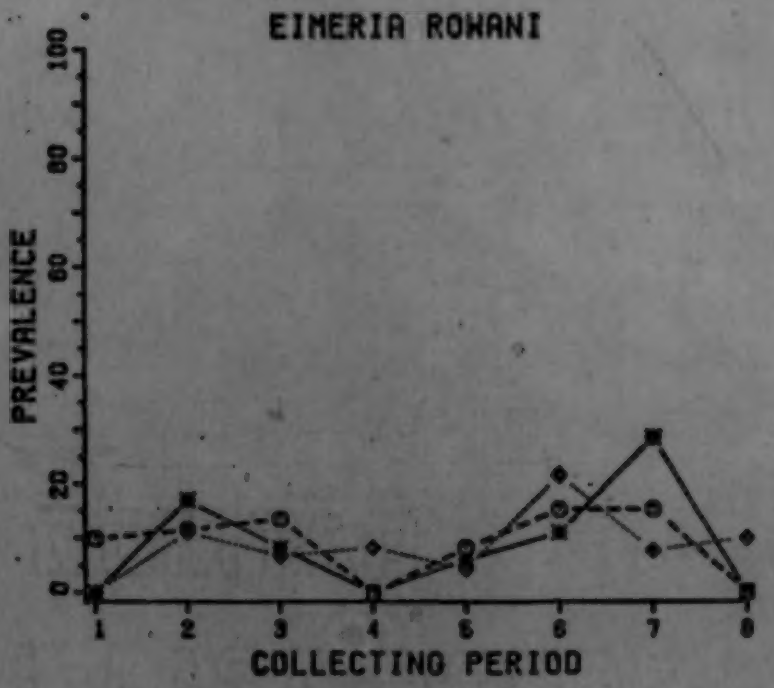
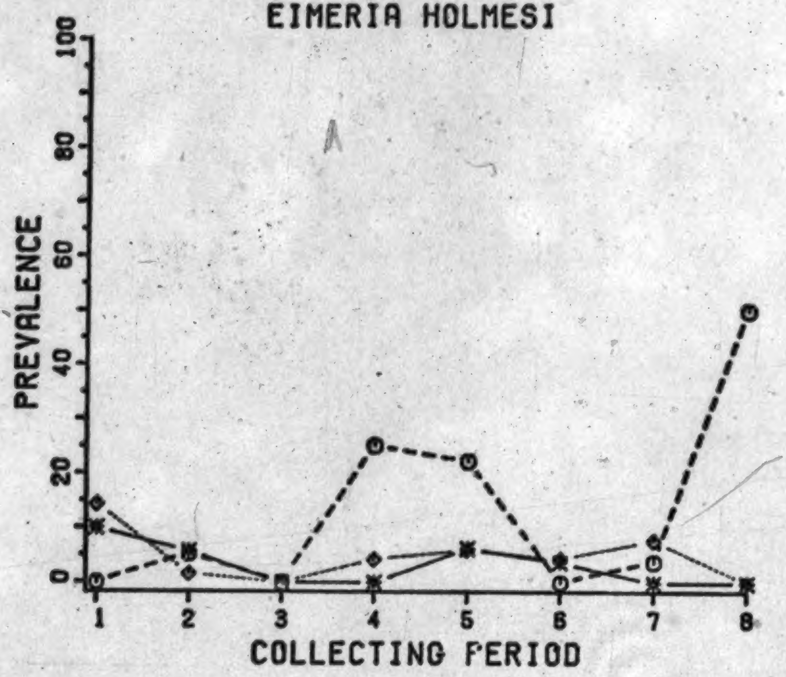

EIMERIA ROWANI
PREVALENCE
0
20
40
60
80
100
1
2
3
4
5
6
7
8
COLLECTING PERIOD

Figure 21. Continued.


EIMERIA HOLMESI
PREVALENCE
0
20
40
60
80
100
1
2
3
4
5
6
7
8
COLLECTING PERIOD

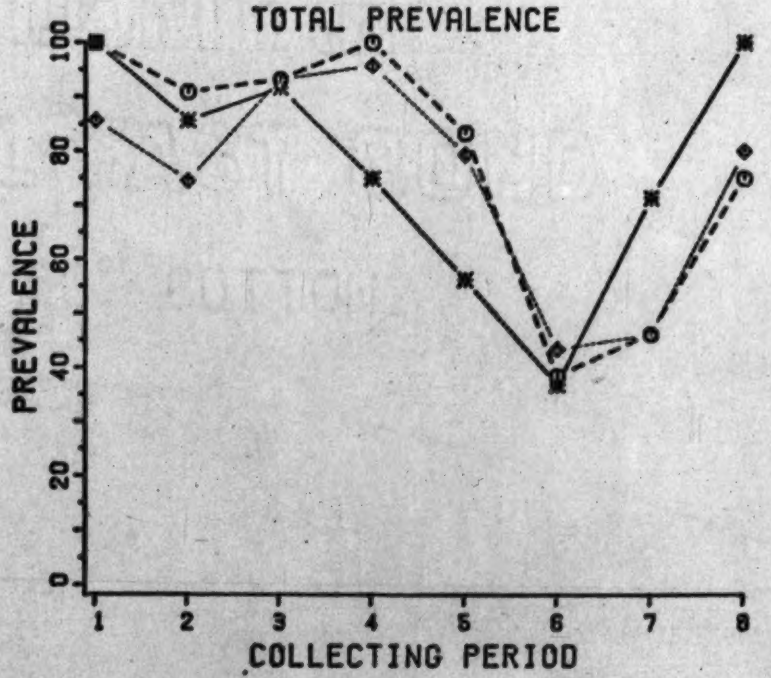
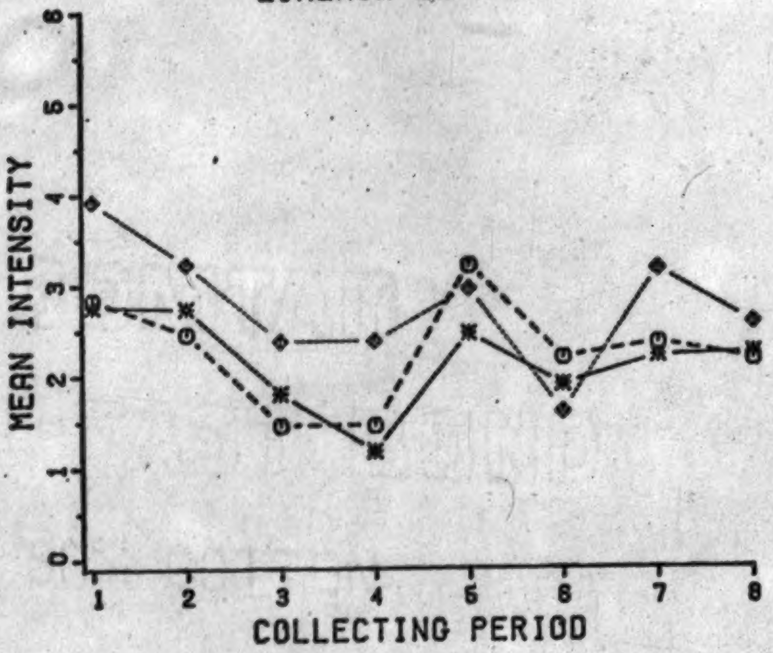

TOTAL PREVALENCE
PREVALENCE
0
20
40
60
80
100
1
2
3
4
5
6
7
8
COLLECTING PERIOD

Figure 22. Seasonal patterns of mean intensity ($\log_{10}$ number of oocysts per gram of feces) in the three age classes, juvenile (—■—), yearling (--○--), and adults (—✦—), of snowshoe hares. Collection periods are listed in Figure 20.

EIMERIA LEPORIS



EIMERIA ATHABASCENSIS

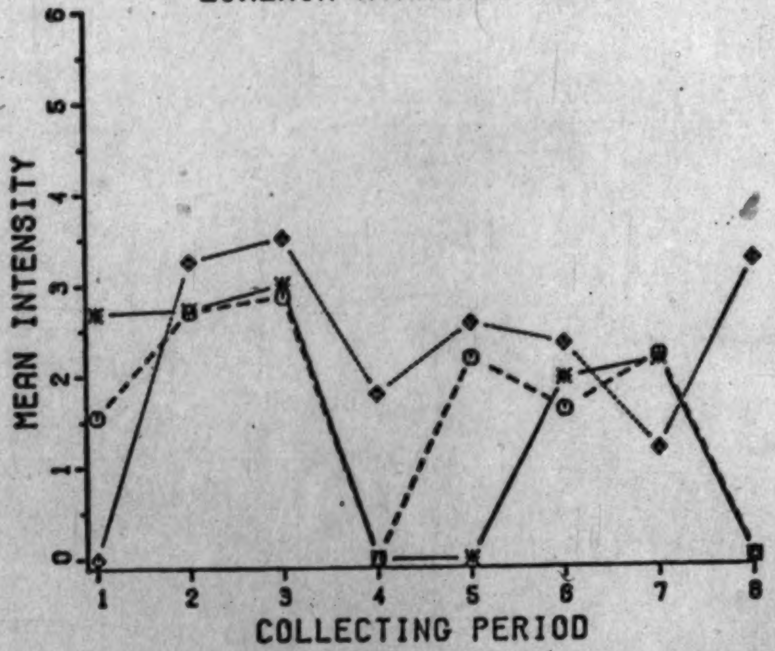
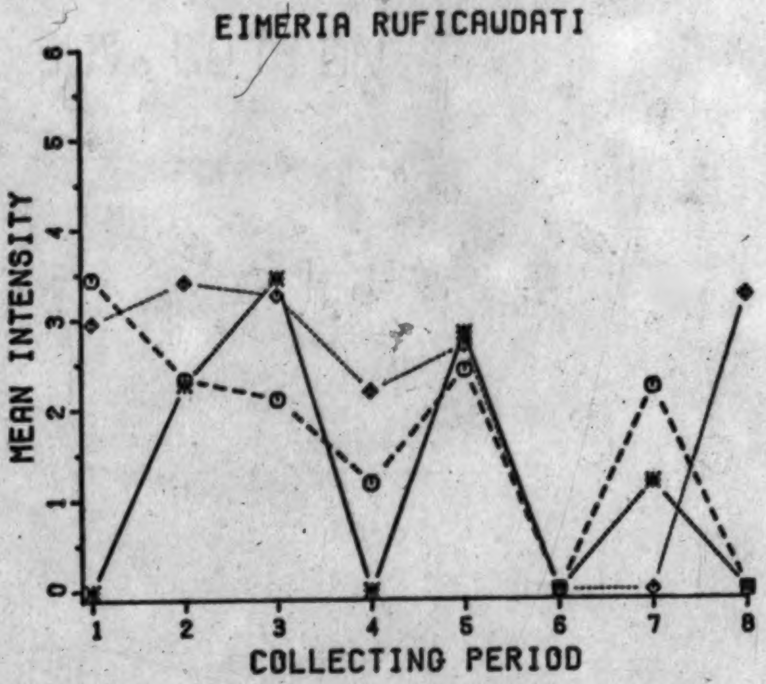


Figure 22. Continued.


EIMERIA RUFICAUDATI
MEAN INTENSITY
0
1
2
3
4
5
6
1
2
3
4
5
6
7
8
COLLECTING PERIOD

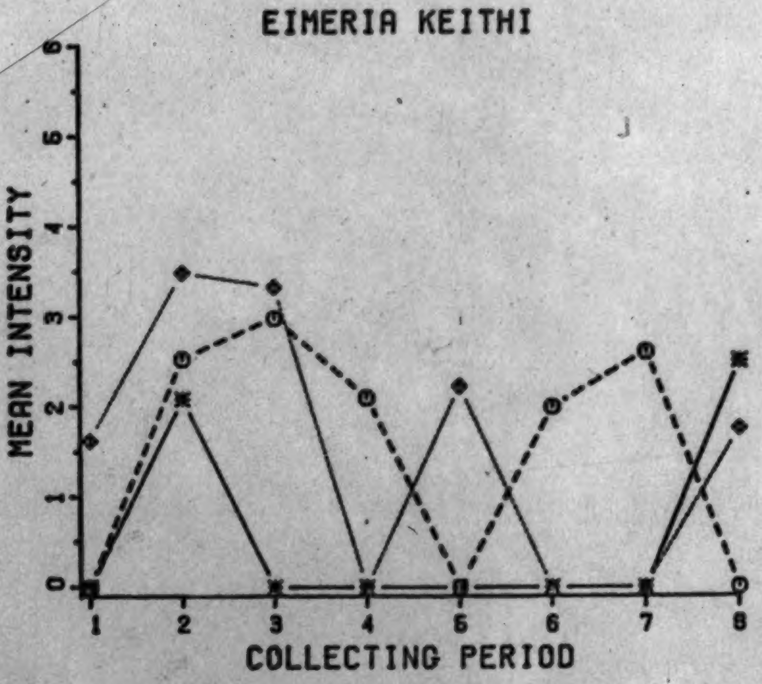
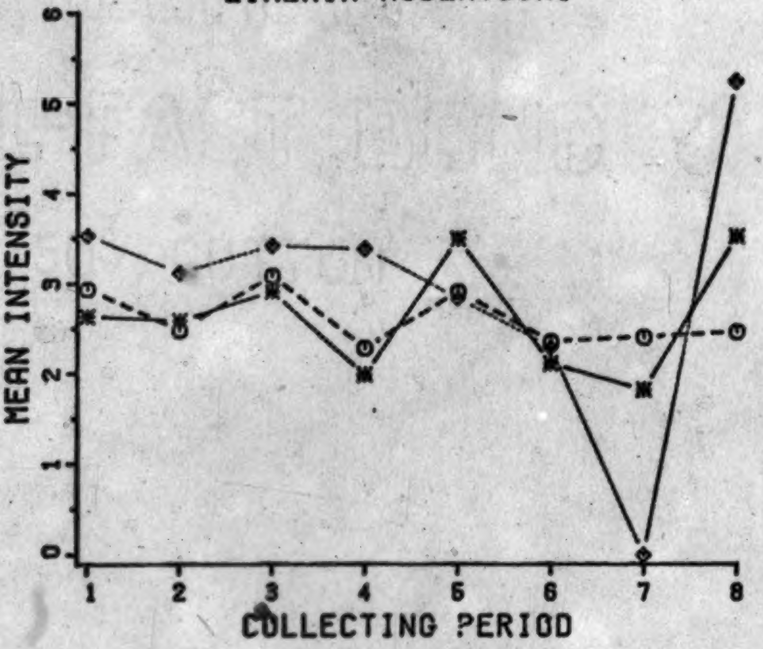

EIMERIA KEITHI
MEAN INTENSITY
0
1
2
3
4
5
6
1
2
3
4
5
6
7
8
COLLECTING PERIOD

Figure 22. Continued.


EIMERIA ROBERTSONI

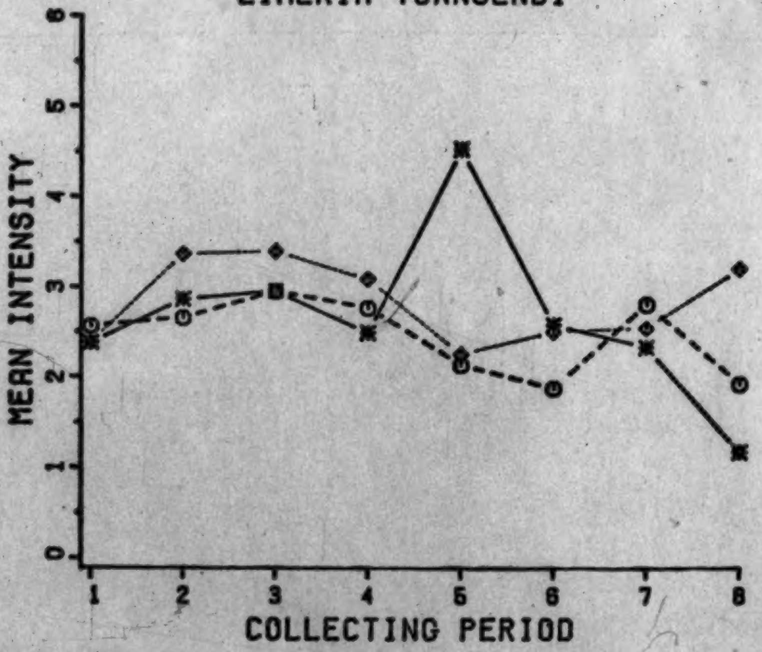
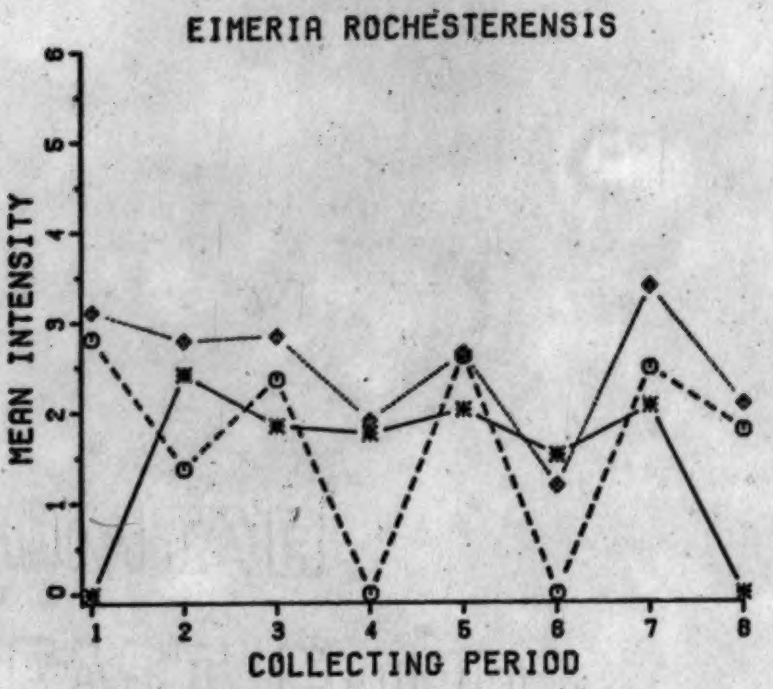
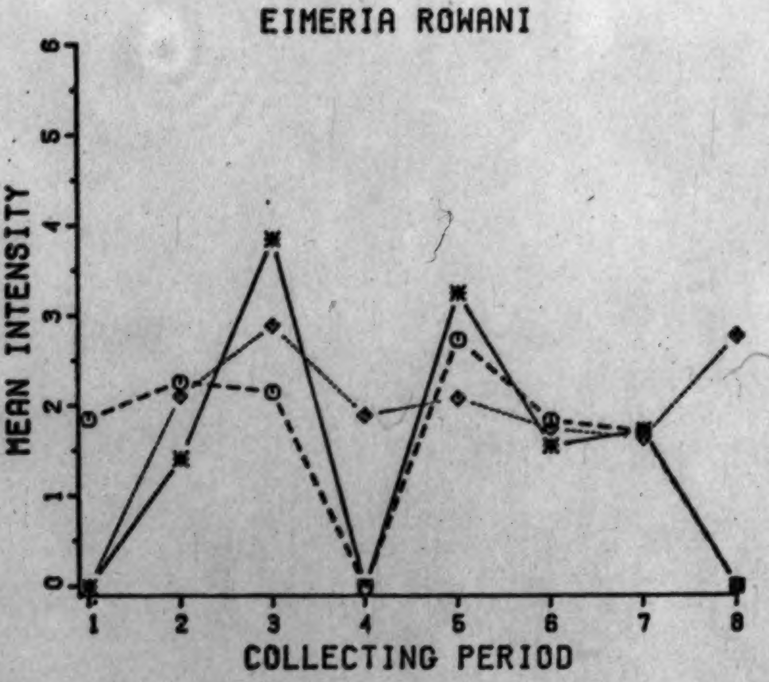

EIMERIA TOWNSENDI

Figure 22. Continued.

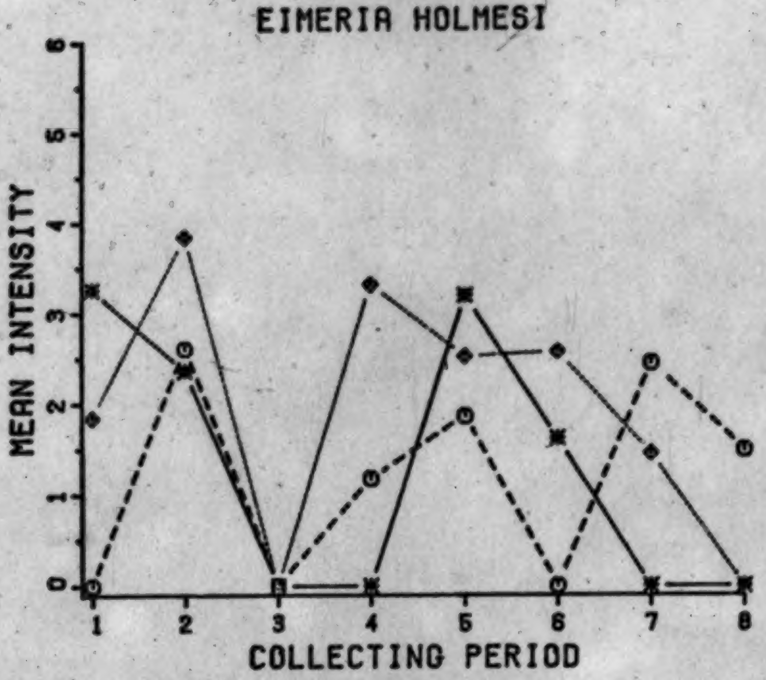


EIMERIA ROCHESTERENSIS



EIMERIA ROWANI

Figure 22. Continued.


EIMERIA HOLMESI
MEAN INTENSITY
0
1
2
3
4
5
6
1
2
3
4
5
6
7
8
COLLECTING PERIOD

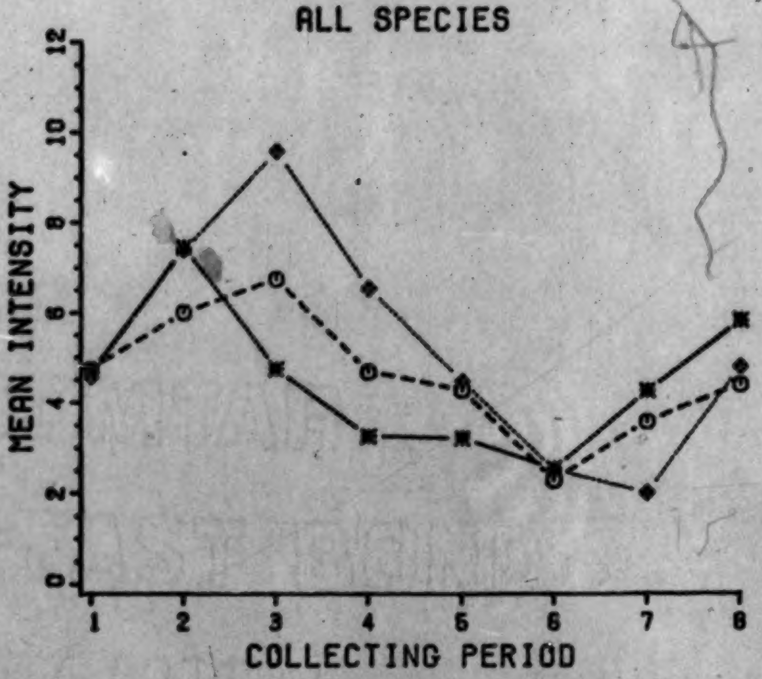

ALL SPECIES
MEAN INTENSITY
0
2
4
6
8
10
12
1
2
3
4
5
6
7
8
COLLECTING PERIOD

Table 12. Per cent prevalence of *Eimeria* spp. in snowshoe hares from 1 to 8 weeks of age

Species of *Eimeria*	Age of snowshoe hares (in weeks)							
	1	2	3	4	5	6	7	8
n*=	82	0	15	19	24	17	14	9
athabascensis	27	-	13	26	38	35	29	78
holmesi	4	-	0	0	0	4	0	0
keithi	4	-	0	21	25	6	7	44
leporis	32	-	20	42	50	53	64	22
robertsoni	24	-	0	21	42	47	36	78
rochesterensis	20	-	13	16	33	41	43	33
rowani	12	-	0	16	4	24	0	22
ruficaudati	12	-	7	26	29	41	36	11
townsendi	43	-	0	21	50	53	79	56

* no. of hares examined

Table 13. Mean intensity of Eimeria spp. in snowshoe hares from 1 to 8 weeks of age

Species of Eimeria		Age of Snowshoe Hares (in weeks)							
		1	2	3	4	5	6	7	8
	n* =	82	0	15	19	24	17	14	9
athabascensis		2.81± 1.00¢	–	2.75± 2.22	3.69± 0.72	3.30±1.00	3.14±1.32	3.91±0.35	3.63± 0.75
holmsei		1.96 ±0.57	–	0	0	0	3.85±0.00	0	0
keithi		3.01 ±1.16	–	0	3.01± 0.91	3.41 ±1.10	3.62±0.00	3.53±0.00	3.72± 0.25
leporis		2.86 ±0.96	–	3.84± 1.01	3.36± 0.74	3.65 ±0.94	3.69±0.79	3.21±0.79	3.28± 1.48
robertsoni		2.97 ±1.15	–	0	3.64±1.11	3.62 ±0.60	3.22±1.10	3.94±1.01	3.45± 0.91
rochesterensis		2.40± 0.71	–	3.25±0.88	3.28± 0.51	3.11 ±0.35	2.94±0.63	2.47±0.76	3.05± 0.72
rowani		2.03±0.86	–	0	2.22±0.91	2.55 ±0.00	2.23±0.66	0	2.72± 0.13
ruficaudata		2.97±0.85	–	4.05±0.00	3.51±0.68	4.11 ±0.23	3.24±0.76	2.94±0.61	3.75± 0.00
townsendi		3.00±1.02	–	0	3.35±1.33	3.40 ±0.85	3.33±0.62	3.52±0.86	4.20± 0.77

* no. of hares examined
¢ $\log_{10}$ number of oocysts per gram of feces   one standard deviation

must be pointed out that the data in this study only cover a sixteen-month period.

Discussion

Some of the factors of the "external" and "internal" environments which have been shown to modify coccidian infections in other lagomorphs are apparently operative on infections in snowshoe hares. Data in this study confirm the results of previous studies (Bull 1958, Mykytowycz 1962, Stodart 1968a, 1971) that increasing age of the animal is generally associated with a marked decrease in oocyst output. Only two of the nine species encountered in this study did not exhibit this relationship, and these two, *E. rowani* and *E. holmesi*, were among the least prevalent and least abundant species encountered.

The association between age and decreased oocyst output has been shown to be due primarily to an immunity derived from previous infections (Rose 1973). Although most eimerians show this type of age relationship, there are some exceptions. Todd and Hammond (1968) reported that three species of ground squirrel (*Spermophilus armatus, S. richarsoni,* and *S. variegatus*) did not develop a resistance to repeated infections of *Eimeria callospermophili*. However, in a subsequent study, Todd *et al.* (1968) found that *S. armatus* and *S. variegatus* could only be infected once with *E. bilamellata*, after which they were refractory to subsequent infections. It is possible, therefore, for a host to harbour species of *Eimeria* which are highly immunogenic and species which appear to elicit little or no immunity. Other reports of a lack of an immune response to species of *Eimeria* are reviewed by Rose (1973).

The role of sex of the host as a modifying factor of coccidian infections was examined. The finding that oocyst output was not related to the sex of the host is in agreement with the findings of Stodart (1968a), who examined the relationship of sex of the host to oocyst output for seven species of *Eimeria* in *O. cuniculus*. In a subsequent study at another site in Australia, Stodart (1971) found oocyst output to be related to the sex of the host for only one (*E. piriformis*) of the seven species of *Eimeria* encountered. Mykytowycz (1962) reported slight differences in total oocyst production related to the sex of the host, females having higher counts than males. Stodart (1971) suggested that, since *E. piriformis* made up almost half of the oocyst count in Mykytowycz's study, the differences were due to the presence of this species. It appears that, for most species of leporid eimerians, sex of the host plays no role in modifying oocyst output.

The seasonal pattern of species richness observed in this study is not surprising based on evidence in the literature. Low temperature and snow cover would be expected to inhibit the sporulation of oocysts and reduce the availability of already sporulated oocysts during the winter period. There is also a seasonal shift in food habits from herbaceous material to woody browse in winter (Dodds 1960), which also reduces the exposure to sporulated oocysts. Therefore, since risk to new infections is reduced and existing infections probably reach the end of their patent period, the number of species per host would be expected to (and did) decrease in winter.

During the spring and summer periods, sporulated oocysts are available to reinfect yearling and adult hares. The relationships between oocyst output and age suggest that older animals may be refractory to some of the species of *Eimeria* while young animals are not, and therefore young animals may be able to acquire more species than older animals during the spring and summer.

The pattern, or lack of it, that is seen in seasonal prevalence and intensity of *Eimeria* species of snowshoe hares suggest that most species can overwinter in hares. The lengths of the patent periods for species of *Eimeria* infecting laboratory-raised leporids ranges from six to twenty-two days (Kheysin 1967, Samoil and Samuel Appendix I), a time period which could not account for the production of oocysts throughout the winter season, a 150-day period.

Dorney (1962) suggested two mechanisms by which coccidian infections could be maintained through the winter: by a lengthening of the patent period; and the possibility of existing areas where the microclimate is suitable for oocyst survival. He suggested (1) the possibility of a positive relationship between stress and patency so that the patent period of an infection would be prolonged under stress, and (2) that oocysts from animals in northern areas are more resistant to cold temperatures than oocysts from southern areas.

When Dorney (1962) made his suggestions about the relationship between stress and the patency, there was little direct evidence to support his contention. Since then, Long and Rose (1970) have shown that treatment with the corticosteroid betamethasone extended the length of the patent period of *Eimeria mivati* in chickens from twelve to forty-five days. The almost four-fold increase in the patent period may be sufficient to explain the seasonal results of the present study; however, more information is needed on the length of the patent periods for the various species of eimerians encountered. Also needed is information on the effect of environmental factors such as temperature on oocyst survival, particularly in the north. Without this type of information, only speculation is possible as to the mechanisms involved.

The final objectives of this study were to examine the relationship between coccidian infections and host density, and to determine if coccidia could be a potential cause of juvenile mortality. Juvenile mortality has been shown to be a factor associated with declining hare populations (Keith 1963). The data collected fail to reveal any clear cut relationships between host density and coccidian infections. However, since these data are only for a sixteen-month period, it is premature to make any conclusions.

Evidence for coccidiosis as a cause of juvenile mortality exists for other lagomorphs (Ecke and Yeatter 1956, Bull 1958, and Dunsmore 1971). Ecke and Yeatter (1956) reported the death of an eight-day-old cottontail (*S. floridanus*) from coccidiosis. Bull (1958) and Dunsmore (1971) presented convincing evidence that hepatic coccidiosis caused by *Eimeria stiedai* was an important factor in juvenile mortality of *O. cuniculus* in Australia and New Zealand. In the present study, animals were infected shortly after birth; oocysts were produced and passed in the feces by the time the hares were one week old. However, there was no evidence that any of the species of coccidia found in this study were pathogenic, although no endeavor was made to study this aspect in detail. The lack of information on the endogenous life cycle of the species found in this study combined with the fact that only live-trapped animals were examined does not preclude the possibility that coccidiosis may be an important mortality factor for snowshoe hares.

VI. GENERAL DISCUSSION

The descriptive phase of the taxonomic work on the eimerians of snowshoe hares is not unique in its approach. What is unique or at least surprising is the lack of study in this area of a most studied host, particularly in view of the implications of other workers (Bull 1958, Dunsmore 1971), for another leporid, *Oryctolagus cuniculus*, that coccidiosis is important in regulating host populations.

The nine species of *Eimeria* described or redescribed here were compared with hundreds of others described previously from lagomorphs, rodents and ruminants. The statistical approach supplements the standard descriptive approach and strengthens the identification of the forms. Selection of oocysts from several host individuals from different collection periods eliminates the potential bias that is so prevalent in the literature when descriptions are based on oocysts from one individual at one point in time (see Levine *et al.* 1967, Sampson 1969, Todd and O'Gara 1969).

The use of parasites as indicators of phylogenetic affinities of the hosts is not unique. What is unique is the use of eimerians, a group which has been ignored in this context, and, unjustly so, according to Metcalf (1929) and Cox (1967). The most interesting aspect of this section of the study is that the use of the criterion of susceptibility of hosts to eimerian infections could be used to confirm suggested phylogenetic affinities.

Beaudoin *et al.* (1970) classified the relationship between parasite prevalence and host-age for several species of helminths and eimerians in white-tailed deer. Class I parasites increased in prevalence with increase in host age; Class II parasites indicated no change in prevalence with host age changes, and Class III parasites decreased in prevalence with increased age. If one assumes that intensity data of *Eimeria* spp. in any host can be classified similarly (Samuel and Trainer 1971, have provided this evidence for deer coccidia) then Class II and Class III curves describes the present host-age data.

The Class II and Class III curves of Beaudoin *et al*. (1970) are analogous to the Type I and Type III patterns of regulation suggested by Bradley (1972). Type I regulation is by variations in the transmission rate through the effects of environmental factors. Type III regulation acts at the individual host level and is by a partial immunity. The patterns observed for the eimerians in this study suggest that their populations are being regulated by the above mechanisms. Two, *E. holmesi* and *E. rowani*, are being regulated by transmission through the effects of environmental factors, possibly temperature and humidity. The patterns for the other seven species suggest that regulation is by a partial immunity.

There are virtually no other studies in which hundreds of wild hosts have been examined for coccidia in North America and with good demographic data, particularly age, provided for each individual. The collection of 629 hares alone could have been a full-time occupation. A team approach with many individuals working to one major end, to know more about the biology of the snowshoe hare, was responsible for getting the present research to completion. Similar approachs with several of the seven other genera of leporids are recommended.

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Appendix I. Experimental study of *Eimeria robertsoni* (Protozoa, Eimeriidae) in the snowshoe hare, *Lepus americanus*: a manuscript in preparation by H. P. Samoil and W. M. Samuel.

ABSTRACT

A 6.5-day pre-patent period and a 22-day patent infection followed single oocyst infection of a young coccidia-free hare with *Eimeria robertsoni*. Size of oocysts increased significantly during patency and was negatively correlated with oocyst output. *Oryctolagus cuniculus* remained negative after inoculation *per os* of *E. robertsoni* of snowshoe hare origin.

Eimeria robertsoni has been reported from five species of *Lepus* (see Levine and Ivens 1972, and Burgaz 1973), but only from the white-tailed jackrabbit (*Lepus townsendii*) of North America. It has not been reported from the snowshoe hare *L. americanus*. Although a few cross-transmission experiments have been attempted with *E. robertsoni* between *L. townsendii* and *Oryctolagus cuniculus* (Carvalho, 1943), *L. europaeus* and *O. cuniculus* (Pellérdy, 1956), and *L. timidus* and *O. cuniculus* (Burgaz, op. cit.), little else is known about the biology of this species of coccidia. The availability of four coccidia-free snowshoe hares (*L. americanus*) reared in the laboratory and oocysts of *E. robertsoni* from snowshoe hares of central Alberta presented the opportunity to study several aspects of the life cycle.

MATERIALS AND METHODS

Pregnant snowshoe hares were live-trapped and brought to the laboratory where the young were born. Neonates were removed from the mother shortly after birth and reared by hand in a coccidia-free environment; they were weaned at 200 grams (4 to 6 weeks). All animals were housed in individual cages with grated floors, kept on a photoperiod of 12 hours light and 12 hours dark at 22 to 25C and provided non-medicated rabbit pellets and water *ad lib*. A single sporulated oocyst of *E. robertsoni*, isolated from hare feces collected in the field, was placed in a gelatin capsule and fed to a six-week old male snowshoe hare. At patency, sporulated oocysts were collected and used in the following cross-transmission experiments: each of two 7-week-old *O. cuniculus* received 100 oocysts *E. robertsoni*; each of four 4-week-old *O. cuniculus* received 50,000 oocysts; and one 2-week-old *O. cuniculus* received 30,600 oocysts (Table 1). A patent infection was established in a previously uninfected "juvenile" *L. americanus* (see Table 1) using the same source of inoculum. One unexposed animal, of the same species, age and sex was maintained in the same room as a control for all experiments.

Feces of all animals were examined three times each week from birth to ensure that they were coccidia-free prior to exposure. After exposure feces were collected and weighed every twenty-four hours, placed in a 2.5% potassium dichromate to give a volume of 1 L, and kept at approximately 20C for seven days. Four separate 2 ml aliquots were placed in test tubes and concentrated for study by flotation in sugar solution (sp. gr. 1.27). A 22 mm^2 coverslip was placed on centrifuge tubes filled to a positive meniscus with the sugar solution and centrifuged for ten minutes at 1,100 rpm. The total number of oocysts per coverslip was determined, the four averaged and the total number of oocysts per twenty-four hours calculated.

One-hundred sporulated oocysts from the single oocyst infection were collected and measured daily starting from day two of the patent period and ending at day nineteen. Measurements were made with a Wild M20 compound microscope using a 10X eyepiece with an ocular micrometer and a 100X oil immersion apochromatic objective. Oocysts were measured in the order in which they were encountered to ensure no prejudicial selection as to size; an oocyst was measured only when it was obvious that its maximum diameter was in view. All measurements are in microns

Table 1. Experimental protocol and results of exposure of *Lepus americanus* and *Oryctolagus cuniculus* to *Eimeria robertsoni* of snowshoe hare origin

Host	Number of hosts	Age of hosts (weeks)	Inoculum (sporulated oocysts)	Source Inoculum	Results
Group I					
*L. a.**	1	6	1	Wild *L. a.*	Positive
L. a.	1	6	none (control)	-	Negative
Group II					
*O. c.***	2	7	100	*L. a.* (Gp. I)	Negative
O. c.	1	7	none (control)	-	Negative
O. c.	4	4	50,000	*L. a.* (Gp. I)	Negative
O. c.	1	4	none (control)	-	Negative
O. c.	1	2	30,600	*L. a.* (Gp. I)	Negative
O. c.	1	2	none (control)	-	Negative
Group III					
L. a.	1	juvenile (18 days)	unknown number	*L. a.* (Gp. I)	Positive
L. a.	1	juvenile (18 days)	none (control)	-	Negative

**L. a.* = *Lepus americanus*
***O. c.* = *Oryctolagus cuniculus*

with means and standard errors in parentheses. Calculation of the geometric mean estimate of the functional regression of Y on X was done according to Ricker (1973).

RESULTS

The inoculation of a juvenile male *Lepus/americanus* with a single oocyst of *E. robertsoni* resulted in a patent infection (Table 1). The pre-patent period was 6½ days and the patent period lasted a minimum of 22 days, at which time the animal was killed.

The daily oocyst output is shown in Figure 1; total output of oocysts was 18,810,255. Oocyst output rose rapidly from 50 on day 1 of patency to over 7,000,000 on day 3, declined steadily until day 9, and thereafter remained near 0. No daily periodicity of oocyst output per weight feces was observed. Oocysts were mainly excreted during the period when 93% of the feces by weight were eliminated (i. e., 9 p. m. to 9 a. m.).

There was a significant increase ($P < 0.01$) in both length (13%) and width (17%) of the oocyst during the eighteen-day patent period (Figure 3). There was a significant negative correlation between oocyst output and both oocyst length ($P < 0.01$, $r = -0.61$), and width ($P < 0.01$, $r = -0.60$). Both oocyst length and width decreased from day 2 to day 3 as oocyst output increased to a peak. As oocyst output declined there was an increase in oocyst length and width.

Cross-transmission attempts, using sporulated *E. robertsoni* from the experimentally infected snowshoe hare and juvenile male *O. cuniculus*

Figure 1. Oocyst output of *Eimeria robertsoni* during the patent period

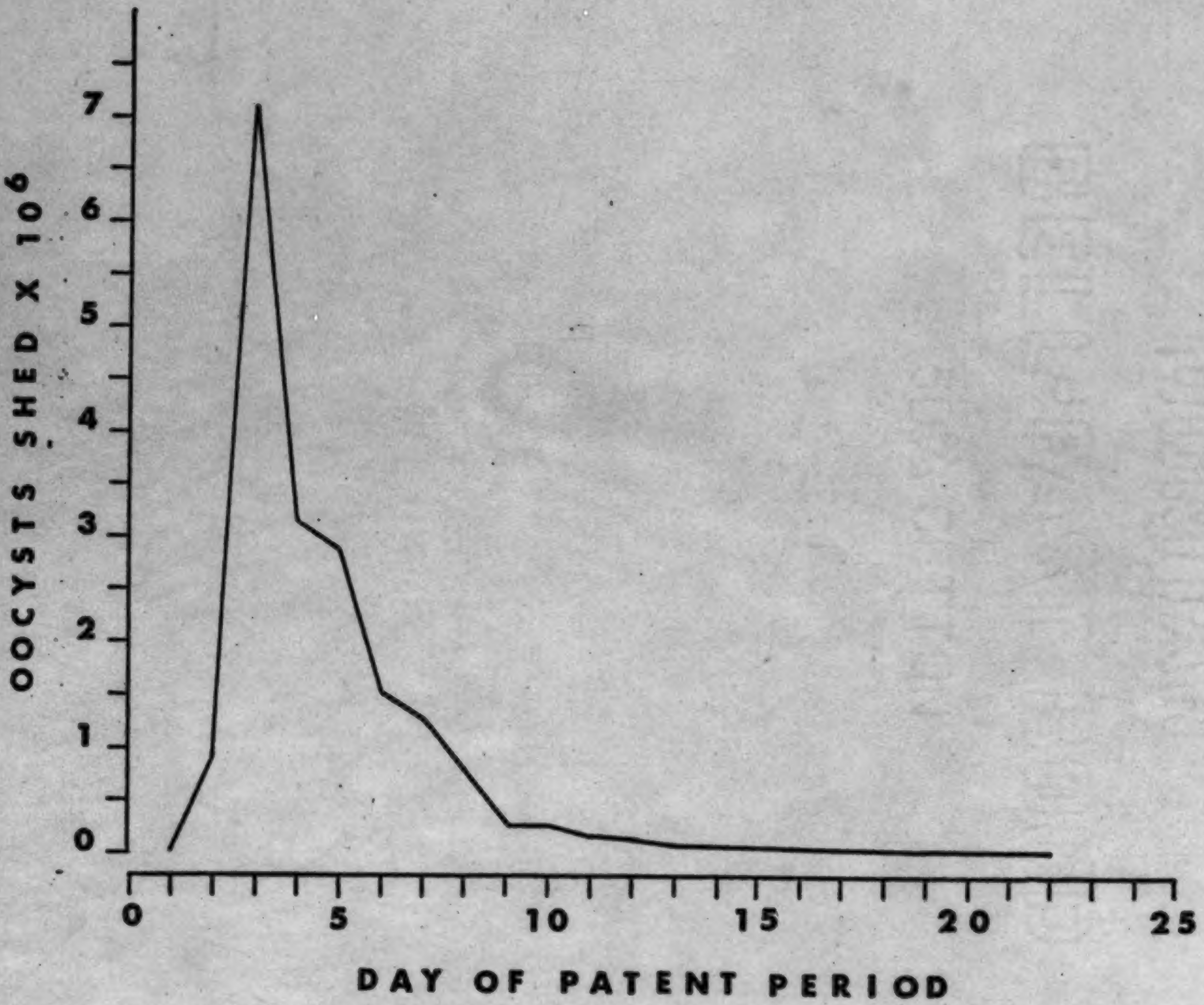
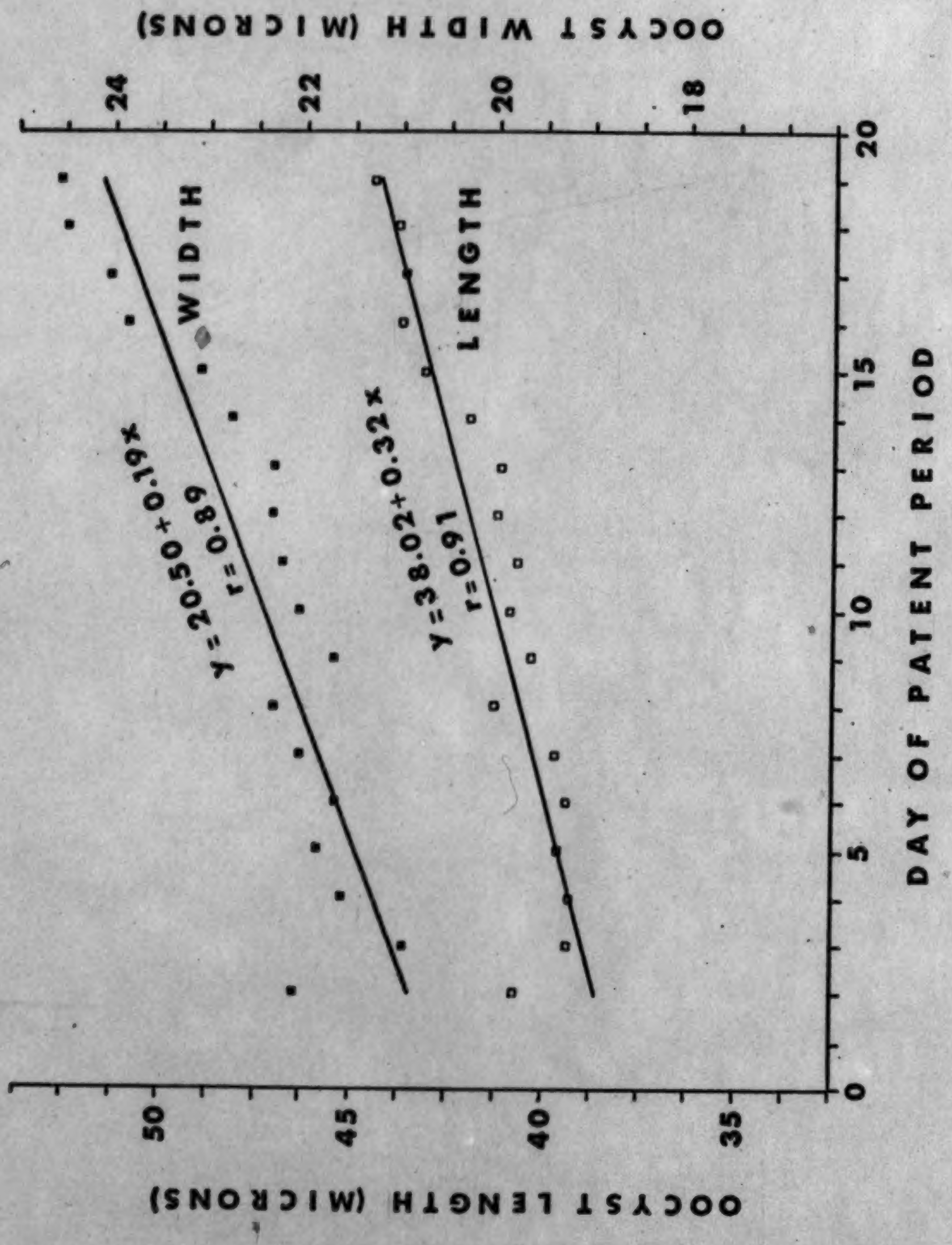

OOCYSTS SHED X 10^6
0
1
2
3
4
5
6
7
0
5
10
15
20
25
DAY OF PATENT PERIOD

Figure 2. Size changes of *Eimeria robertsoni* during the patent period


OOCYST WIDTH (MICRONS)
24
22
20
18
WIDTH
Y = 20.50 + 0.19x
r = 0.89
LENGTH
Y = 38.02 + 0.32x
r = 0.91
OOCYST LENGTH (MICRONS)
50
45
40
35
0
5
10
15
20
DAY OF PATENT PERIOD

as recipients, were unsuccessful (Table 1). Seven *O. cuniculus* remained negative despite receiving up to 50,000 sporulated oocysts.

DISCUSSION

Dimensions and morphology of the oocyst are characteristics most commonly used to describe species of coccidia. Most characters remain constant over time, however, length and width of oocysts of several species vary (see Discussion of Duszynski 1971). Briefly, results of the present study confirm those of Fish (1931) for *E. tenella* from chickens, Kheysin (1947) for *E. magna* in rabbits, Becker *et al.* (1955) for *E. brunetti* in chickens, Becker *et al.* (1956) for *E. necatrix* from chickens, and Duszynski (1971) for *E. separata* in rats, that progressive changes in oocyst length and width during the patent period.

Kheysin (1967) felt that crowding of macrogametes in one host cell and the influence of host diet were the major factors which might contribute to a change in oocyst size. Jeffers (1975) stated that oocyst size was a function of the time permitted for growth of the macrogametes. Regardless of the reasons accounting for this phenomenon, caution is needed in the identification and description of species of coccidia such as *E. robertsoni* with major features such as length and width of the oocyst and sporocyst residuum which vary in size during storage.

Cross-transmission experiments with *E. robertsoni* have been unsuccessful. Carvalho (1943) was unable to infect either the tame rabbit (*O. cuniculus*) or the cottontail (*Sylvilagus floridanus*) with *E. robertsoni* from the jackrabbit (*L. townsendii*). Pellérdy (1956) could not infect *O. cuniculus* with *E. robertsoni* from *L. europaeus*, and Burgaz (1973) was unable to infect *O. cuniculus* with *E. robertsoni* from *L. timidus*. Our negative results tend to confirm the specificity of this coccidian for the genus *Lepus*.

ACKNOWLEDGMENTS

M. Bush, R. Hobbs, R. McClymont, D. Wing, and graduate students and support staff of the University of Wisconsin Wildlife Research Center at Rochester, Alberta, assisted with the study. This study was supported, in part by the Boreal Institute for Northern Studies, the National Research Council of Canada (Operating Grant A-6603), and the Alberta Fish and Wildlife Division.

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